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Sound Localization in Reverberant Environments: Physiological Bases of the Precedence Effect

By

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Abstract

Localization dominance, a phenomenon of the precedence effect, refers to the dominance of directional cues conveyed by sound arriving directly from the source over cues conveyed by reflected copies on the perception of sound source location. One theory of localization dominance is that leading sounds suppress neural responses to lagging sounds (Yin, 1994; Litovsky & Yin, 1998 a, b). Neurons in auditory nuclei respond best to a leading sound and have a reduced response to a lagging sound, supporting this hypothesis. It has been proposed that GABA-ergic or glycinergic inhibition suppresses neural responses to lagging sounds (Yin, 1994; Fitzpatrick at al. 1995; Pollack & Burger, 2002). An alternative hypothesis states that cochlear processing in low-frequency hearing animals alters directional cues conveyed by the leading and lagging sound, emphasising those present in the leading sound (Tollin, 1998; Hartung & Trahiotis 2001). Responses of single neurons in the inferior colliculus (IC) of anaesthetised guinea pigs were recorded to binaural click pair stimuli. Responses of some neurons were recorded before, during, and after iontophoresis of either the $GABA_A$ receptor antagonist gabazine, or the glycine receptor antagonist strychnine. Blocking glycine did not decrease neural suppression of the lagging click in 8/10 neurons. Blocking GABA did not decrease neural suppression of the lagging click in 11/16 neurons. The neural representation of directional cues in the output of low-frequency neurons to the leading click of a binaural click pair differed from those actually conveyed by the stimulus in 20/20 neurons. Examination of the responses of several such neurons indicated responses to the leading click represented a direction between that conveyed by the leading and lagging click. The results supported the hypothesis that cochlear processing of binaural click pairs alters directional cues conveyed by the stimulus. Limited support was also found for the hypothesis that GABA-ergic and glycinergic suppress lagging click responses in some neurons.

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1.0 Introduction

Listeners are capable of accurately localizing the source of a sound using directionally dependent acoustical cues, which result from the sound's physical interaction with the listener. The two primary directional cues used by human listeners to localize sound are the interaural time difference (ITD) cue and the interaural intensity difference (IID) cue. ITDs and IIDs require comparison between the sound arriving at the two ears and, as such, are known as binaural cues. ITDs result from differences between the arrival of the sound at the ear nearest the source (near ear) and the ear farthest from the source (far ear). As the location of a sound source changes so too the ITD changes, providing unique ITDs for a range of sound source locations. IIDs arise when sound is reflected away from the surface of the head closest to the sound source, resulting in the sound reaching the far ear being less intense than that reaching the near ear. The IID cue, like the ITD cue, is dependent on the angle of incidence of a sound with the head, and is unique for a range of sound source locations. Sound localization in humans is dominated by the ITD cue for sounds containing frequencies below 2.5-kHz and by the IID cue for sounds containing only frequencies above ~ 5-kHz (Wightman & Kistler, 1992). Localization of low-frequency sounds utilising ITD cues, and of high-frequency sounds utilising IID cues, is referred to as the duplex theory of sound localization (Raleigh, 1907; Additional information about sound localization cues is provided in Appendix A).

In circumstances where a listener is exposed to a single sound arriving directly from the source, sound localization cues provide unambiguous information about a sound's origin. However, surfaces in a listener's environment reflect sounds, exposing them to multiple copies of each sound. Reflected sounds that are not heard as echoes are referred to as reverberation. Despite acoustic energy arriving at different times from multiple locations however, listeners hear only a single sound and are able to locate the sound source with a degree of accuracy. The underlying behavioural phenomenon that allows listeners to hear only a single sound and localize it accurately under these conditions is known as the precedence effect (Wallach et al. 1949).

Neurophysiological studies have found that when presented with the same stimuli used in psychophysical studies of the precedence effect, temporal patterns observed in the

output of single neurons located in the mammalian and avian auditory pathways correlate with behavioural measures of the precedence effect (Yin, 1994; Fitzpatrick et al. 1995; Spitzer et al. 2004). The physiological mechanisms thought to underlie neural correlates of the precedence effect in species that rely on low-frequency directional information to localize sound, such as humans, remain to be determined.

1.1 The Precedence Effect

In acoustically-reverberant environments listeners can extract unambiguous information about a sound despite the ears receiving not only the sound direct from the sound source, but also a number of potentially confusing reflected copies arriving milliseconds later (Figure 1.1.1). Under such conditions, listeners hear only a single sound and assign the location near to that indicated by the direct sound, seemingly ignoring the directional information in the reflections. As a result, sound localization in reverberant environments remains accurate. The dominance of the directional information in the first-arriving wave front on the perception of sound is referred to as the 'precedence effect'.

In the definitive study concerning the precedence effect, Wallach, Newman and Rosenzweig (1949) described the stimulus and listening conditions under which the precedence effect occurs and, by identifying behaviourally-relevant stimulus parameters, introduced terms commonly used to describe aspects of the effect. Wallach et al. (1949) defined the period during which the listener hears only a single sound despite the presence of multiple copies of that sound as **fusion.** They considered this a requisite of the precedence effect. To specify the upper limit of fusion the term **echo threshold** has since been introduced. Wallach et al. (1949) referred to the dominance of the directional information contained in the leading sound over that in the lagging sound as the precedence effect in sound localization. As such, the phenomenon as it was originally defined referred unambiguously to binaural sound localization cues. However, more recent studies have indicated that the dominance of the directional information present in the leading sound and fusion may be independent phenomena (Clifton et al. 2002). In addition the leading sound can also suppress aspects of the lagging sound other than its location (Aoki & Houtgast 1992; Houtgast & Aoki, 1994). Therefore, in more recent studies the term **localization dominance** is used to specify the dominance of the directional information conveyed by the leading sound and to distinguish it from the phenomenon of fusion. Wallach et al. (1949) also identified another period that occurs prior to localization dominance when the interval between the leading and lagging sound is very short (< 1 ms). During this period, referred to as **summing localization** (Bekesy, 1930), the listener hears the fused sound originating between the locations indicated by the leading and lagging sound.

In their first series of experiments Wallach et al. (1949) reproduced reverberant listening conditions by presenting listeners seated in a large room with a leading sound, representing sound arriving directly from the source, and a single lagging sound (a delayed copy of the leading sound), representing a reflection of equal amplitude. The leading and lagging sounds were delivered from separate speakers with distinct locations equidistant from the listener. Listeners were presented with clicks, continuous tones, human speech, piano music or orchestral music. Listeners experienced fusion of the leading and lagging sounds for intervals (lead-lag intervals) of up to 35 ms during which localization dominance occurred. Wallach et al. (1949) found localization dominance was greatest for clicks, speech and piano music and absent for continuous tones and orchestral music. From this they concluded that sounds containing fast changes in amplitude, or spectral content that result in transients were more effective in eliciting fusion and therefore localization dominance.

In a second series of experiments Wallach et al. (1949) went on to further describe fusion and localization dominance by presenting listeners with binaural click pairs over headphones (closed field listening conditions). By so doing, the possibility of additional lagging sounds arising from unwanted reverberation extending the period of fusion could be ruled out. The perceived location of the leading and lagging clicks was manipulated by imposing interaural time differences (ITDs) on them. Altering the ITD of a binaural click presented over headphones results in the perception of the click changing position within the listeners head toward the ear at which the first click arrives. By convention the term sound lateralization rather than sound localization is used when describing head phone experiments as the sound moves only from left to right and is not perceived as external to the listener. Wallach et al. (1949) set the lead-lag interval (LLI) at 2 ms, which was below the lower limit of fusion under closed field conditions for those listeners (3-6 ms) and varied either the ITD of the leading or lagging click (see figure 1.1.2). They asked listeners to judge from which side of the head the sound originated. When the ITD of the lagging click was held constant and the ITD of the leading click altered, listeners judged the fused clicks to originate from the side of the head indicated by the leading click although judgments were biased in the direction indicated by the ITD of the lagging click. When the ITD of the leading click was held constant and the ITD of the lagging click altered listeners judged the fused sound to originate from the side of the head indicated by the leading click although

biased in the direction of the lagging click. To examine the bias on judgments of laterality caused by the lagging click ITD, Wallach et al. (1949) altered the ITD of the leading click between -100 μs and +100 μs and asked listeners to centre the image by adjusting the ITD of the lagging click. They found listeners could centre the fused sound by adjusting the ITD of the lagging click to extreme values (~ 4 times that of the lead ITD) which indicated the opposite side of the head to that of the leading click ITD.

The study by Wallach et al. (1949) addressed two salient issues associated with sound localization in acoustically-reverberant environments; (1) the phenomenon of fusion which concerns the acoustical qualities used by a listener to differentiate between reverberation, echoes and different sounds, and (2) the phenomenon of localization dominance which concerns the degree of dominance of the leading sound and the cues which a listener then relies on to localize the fused sound. The findings of Wallach et al. (1949) showed that for fusion to occur, the interval between leading and lagging sounds must be sufficiently short. They showed that a sound's spectrum was important, since fusion occurred most readily for clicks and sounds containing transients. They also described the necessity of qualitative similarity between leading and lagging sounds for the precedence effect to occur. They emphasized the finding that localization dominance was not complete, and altering the ITD of the lagging click to extreme values could result in substantial shifts in the lateral position listeners perceived fused binaural click pairs to originate from. They also stated that the precedence effect could be disrupted by increasing the amplitude of the leading sound (Langmuir et al. 1944).

1.1.1 Factors mediating stimulus fusion

Later studies provided support for the echo thresholds determined by Wallach et al. (1949) for binaural click pairs (3-6 ms) and speech (35-70 ms). Echo thresholds depend partly on the criteria used to define an echo and are variable between listeners (Litovsky & Colburn, 1999). Regardless of this, however, many studies indicate that for most listeners the break down of fusion for binaural click pairs begins at LLIs of 5 ms and is complete for LLIs greater than 10 ms (Ebata et al. 1968; Freyman et al. 1991; Yang & Grantham, 1997a, 1997b; Litovsky et al. 1999). Other measurements of the echo threshold for continuous speech have also proven to be within the range identified

by Wallach et al. (1949) reported as extending up to 50 ms (Haas, 1951; Lochner & Burger 1958). Furthermore, echo threshold has been directly linked to the duration of the sound with the echo threshold for paired noise bursts shown to increase with stimulus duration (Ebata et al. 1968; Schubert & Wernick, 1969). Wallach et al.'s (1949) suggestion that the presence of reverberation may extend echo thresholds has also been validated (Ebata et al. 1968; Roberts et al. 2004).

The role of spectral similarity between the leading and lagging sound in mediating the precedence effect identified by Bekesy (1930) has also been investigated. Perrott et al. (1987) showed that when two noise bursts from independent sources (uncorrelated) were presented to listeners via separate loudspeakers with an LLI of 2 ms listeners perceived two distinct sounds at their respective locations. When the lagging noise burst was a copy of the leading noise burst (correlated) listeners perceived only a single sound originating at the location of the speaker from which the leading noise was delivered. A similar result is obtained for leading and lagging sounds with different spectra. When listening to trains of broadband noise bursts delivered from separate speakers, listeners perceive the noise originating from the speaker of the leading noise; narrowing the spectrum of the lagging sound results in the breakdown of fusion, the lagging sound being localized at its respective speaker (McCall et al. 1998).

The importance of the relative sound intensities in mediating fusion and localization dominance (Langmuir et al. 1944) has been recently confirmed. Increasing the intensity of the leading sound relative to the intensity of the lagging sound has been shown to extend echo threshold in human listeners when listening to pairs of noise bursts (Roberts et al. 2004), whereas decreasing it has been shown to reduce echo threshold (Hass, 1951). Furthermore, both echo threshold and localization dominance in rats localizing paired stimuli have been shown to increase when the leading sound is more intense (Hoeffding & Harrison, 1979). Interestingly, the overall intensity of the leading and lagging sounds is also important, with the precedence effect breaking down at low stimulus intensities (Goverts et al. 2000; Saberi et al. 2004).

1.1.2 Factors Mediating Localization Dominance

Localization dominance reflects the dominant contribution of the directional cues in the leading sound to listeners' localization judgments of fused sounds. The directional cues in the lagging sound however are not completely ignored. As stated by Wallach et al. (1949) when discussing the slight bias of listeners' lateralization judgments toward the direction indicated by the lagging sound "Under proper conditions the second sound can be shown to have a quite small yet demonstrable effect." A complete replication of the Wallach et al. (1949) study using a greater number of listeners (Yost & Soderquist, 1984) and several other studies (Blauert, 1982; Fitzpatrick et al. 1999) have confirmed the contribution of the directional cues conveyed by the lagging sound to listeners' localization judgments during fusion.

The strength of localization dominance is largely determined by the same factors mediating fusion. The spectral similarity of the leading and lagging sound determines the degree of dominance of the directional information in the lead sound i.e. lagging sounds that are composed of frequencies present in the leading sound are more suppressed than those that aren't (Yang & Grantham, 1997). The relative level of the leading and lagging sounds (Langmuir, 1944; Hoeffding & Harrison, 1979) and the absolute level of both the leading and the lagging sound, which have been shown to mediate fusion (Goverts et al. 2000; Saberi et al. 2004), also affect the degree of localization dominance in the same manner.

The frequency composition of the leading sound has also been shown to influence localization dominance. Experiments designed to assess the degree of dominance of the leading sound often require listeners to discriminate changes in the lagging stimulus. Several studies have shown that, when presented with narrow-band noise bursts over headphones, listeners are less able to discriminate changes in the ITD of the lagging noise when the leading noise is centred below 2 kHz (Divenyi, 1992; Shinn-Cunningham et al. 1995; Yang & Grantham 1997). The interpretation applied to this finding was that localization dominance was determined by the localization 'strength' of the leading noise; listeners were more sensitive to changes in the laterality of low-frequency narrow-band noise than high-frequency narrow-band noise (Divenyi, 1992; Shinn-Cunningham et al. 1995). This finding was replicated for stimuli presented in the

free field in which the localization strength of the noises was manipulated by altering the speed of their onset. Listeners' localized single noise bursts with faster onsets more accurately than those with slower onsets. As a result, localization dominance was greater for leading sounds that had faster onsets (Yang & Grantham 1997). Additionally, Tollin and Henning (1999) have shown listeners localization judgments of fused click pairs to be predicted by the localization cues present in the stimulus frequencies surrounding 700 Hz, suggesting that ITD cues are the most salient even when the stimuli are broad band and high-frequency directional cues are available.

1.1.3 Other Aspects of the Dominance of a Leading Sound

A number of studies have addressed the question of whether the suppressive effect of the leading sound on perception of the lagging sound extends beyond binaural localization cues. By manipulating the correlation between the noise delivered to the left and right ear the spatial extent of the sound can be altered; uncorrelated signals sound more diffuse in terms of laterality than correlated signals. Listeners' perception of the spatial extent of fused pairs of noise bursts delivered via headphones is determined by their perception of the spatial extent of the leading sound (Aoki & Houtgast, 1992; Houtgast & Aoki, 1994). It should be pointed out that the manipulation of the interaural correlation will affect ongoing ITD cues, and as such, changes to the spatial extent while not pertaining directly to localization cues, arise through manipulation of those cues.

Litovsky et al. (1997) have shown that the precedence effect also operates in the mid-sagittal plane where there is an absence of conflicting binaural cues between the lead and lag sounds. Their finding suggests that spectral cues that are considered monaural, and thus provide cues to sounds source elevation and front-vs.-back location, are also suppressed by the precedence effect. This appears to rule out precedence as a strictly binaural phenomenon, suggesting rather that it is a more general directional phenomenon. This interpretation may not be entirely accurate however, as listeners localizing sounds in the mid-sagittal plane have access to two sets of monaural spectral cues (one for each ear). This may therefore be interpreted as an interaural spectral difference cue and not a strictly monaural cue. Listeners utilising monaural spectral

cues from a single ear in a sound localization task perform little better than chance (Martin et al. 2004).

1.1.4 Is There Adequate Evidence for Suppression of the Lagging Sound?

Pervasive in the literature concerned with the precedence effect is the notion that the leading sound suppresses the localization cues conveyed by the lagging sound. This implies that localization cues are absent from the auditory systems representation of the lagging sound. While there is evidence that listeners' perception of fused sound pairs is largely predicted by their perception of the leading sound alone, there is also much evidence pointing to the adequate representation of the lagging sound in the auditory system.

Reflected sound is known to contribute to the perceived spatial extent of a fused sound, making the sound appear as if it emanates from a larger area of space, a quality desirable in concert halls (Zurek, 1979; Blauert, 1997 pp 348-349). Speech comprehension also benefits from the presence of a single reflection as it results in an increase in the intensity of the speech due to additional energy provided by the reflection (Haas, 1972). Furthermore, listeners presented with a train of leading and lagging binaural click pairs perceived a single train of clicks originating from the location indicated by the leading click pair. These listeners experienced a breakdown of fusion if the ITD of the lagging click was changed, hearing instead two distinct binaural clicks originating from their respective locations. A change in the ITD of the lagging click would indicate a new reflective surface, i.e. a change in the room or the position of objects in the room, indicating listeners use reflections to inform them of the physical attributes of their environment and do not suppress them (Clifton, et al. 1994; McCall et al. 1998; Clifton at al. 2002). Listeners are also equally sensitive to intensity changes of the leading and lagging clicks (Freyman et al. 1998) suggesting that perception of level is not dominated the leading sound. All of the above instances indicate that while the lagging sound is not perceived as a separate auditory event it contributes to listeners perception of fused sounds and is seemingly not suppressed. This extends to directional cues in the case of listeners using reflections to determine the characteristics of their environment. It is questionable then that any aspect of the lagging sound is removed from the auditory systems representation of fused sounds.

1.3.3 The Precedence Effect Summarised

The combined findings concerning the precedence effect lend themselves to the following description of the phenomenon. When two sounds of similar enough spectral characteristics arrive in quick succession they are perceived as a single sound (fusion). The lagging sound contributes substantially to listeners' perception of the fused sound altering its loudness, spatial extent and timbre relative to the leading sound presented in isolation. The perceived location of the fused sound is determined by a temporal weighting of the directional information in the fused sound, with greater weight appointed to the leading sound (localization dominance). Significant alterations to the intensity, spectrum or, to a lesser extent, directional cues present in the lagging sound can result in disruption of fusion, localization dominance or both.

Within the literature, the term echo suppression is often used to describe the phenomena of fusion and localization dominance. The term 'echo suppression' implies that the lagging sound provides redundant and potentially confusing information to the listener and as such is made absent from the auditory systems representation of the fused sound. Given the contribution of the lagging sound to the perception of the fused sound, and the possibility that the directional information contained in the lagging sound is still available to the listener under specific circumstances (Clifton et al. 2002) such a term must be used with caution. It appears echo suppression would only apply to specific auditory pathways dedicated to the processing of directional cues in a limited way.

An alternative interpretation of the precedence effect to one of echo suppression is possible from the available literature. The naïve auditory system is not confronted with echoes i.e. reflected sound originating from a single source, but a series of sounds of extremely similar spectral characteristics originating from different locations and arriving at different times that effectively indicate different sources. If reflections are conceptualized as separate sound sources the fusion of reverberated sound with the sound arriving direct from the source reflects the limitations of the system in separating sounds arriving from different locations in space that have very similar spectral characteristics.

Our understanding of the precedence effect resulting from the experiments described could be formulated as such; when successive sounds arrive at the ears from different locations we can separate them if; a) they are separated sufficiently in time from the direct sound, b) their spectrum differs enough from that of the sound from the source i.e. if they are a different sound, and c) they are intense enough relative to the intensity of the leading sound. It is not possible, however, to separate them on the basis of their spatial location using individual directional cues (ITDs or IIDs manipulated in closed field studies) or all available directional cues (free field studies). Localization dominance then describes the way in which listeners utilize available directional cues from the leading and lagging sound to locate fused sounds.

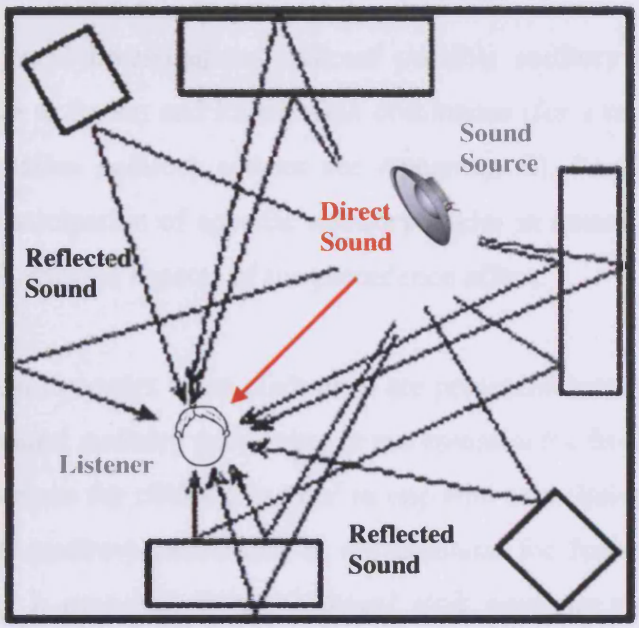


Figure 1.1.1 Reverberations resulting from sound in a room with many reflective surfaces. The reverberations have longer path lengths to the listener than the direct sound and as such arrive later. In this circumstance the listener would hear a single sound originating from the location of the sound source.

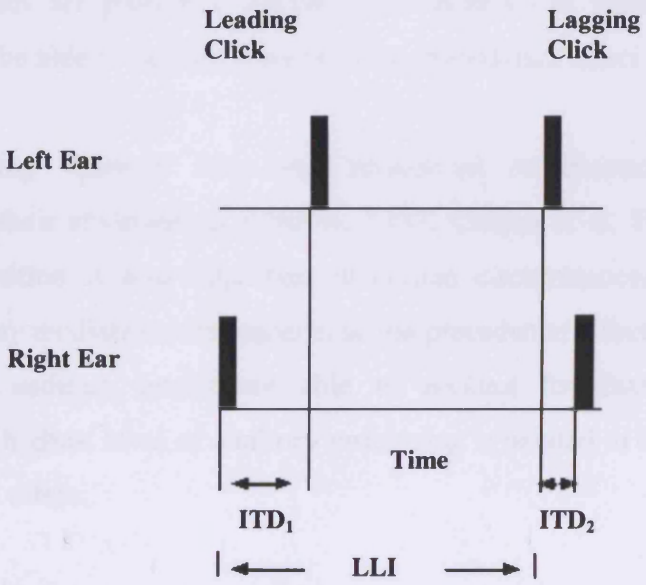


Figure 1.1.2 Binaural click stimuli commonly used in studies of the precedence effect. Directionality is imposed on the leading and lagging clicks by introducing an ITD. The three parameters often varied are the ITD of the leading and lagging clicks (ITD_1 & ITD_2) and the interval between them (lead-lag interval or LLI).

1.2 The Physiological Basis of the Precedence Effect

A number of psychophysical investigations indicate possible auditory pathways and nuclei that may contribute to fusion and localization dominance (for a review of neural processing in the mammalian auditory system see Appendix B). Results from such studies necessitate the participation of specific auditory nuclei in neural processing to account for particular behavioural aspects of the precedence effect.

It has been shown that fusion occurs when click pairs are presented monaurally (Zurek, 1979) indicating that binaural auditory processing is not essential for fusion. It has also been shown that fusion occurs for clicks presented to opposite ears (Babkoff & Sutton, 1965) showing monaural auditory processing is not essential for fusion and, in this case, binaural processing is essential. Where binaural click pairs are presented, echo thresholds increase by ~ 2 ms relative to monaural presentation or presentation of single clicks to each ear (echo threshold ~ 3 ms), indicating binaural fusion of binaural click pairs is different to monaural fusion or binaural fusion of single clicks. The increases in echo threshold when binaural click pairs are presented relative to when monaural click pairs are presented indicates the necessity of processing by binaural auditory nuclei to be able to explain aspects of the precedence effect.

Research indicating listeners may use reflections to determine the physical characteristics of their environment (Clifton, 1987; Clifton et al. 1994; Clifton, et al. 2002) imply cognition is also important in certain circumstances and as such the auditory cortex may mediate certain aspects of the precedence effect. So it appears that while the lower auditory nuclei are able to account for fusion, under certain circumstances the highest level of auditory processing is needed to completely account for the precedence effect.

Temporal weighting of the leading sound evident in localization dominance could be achieved through neural adaptation or inhibition occurring in either the monaural or binaural auditory pathways (Zurek & Saberi, 2003). The greater dominance of the directional information of a leading sound rather than other aspects such as the level of the lagging sound (Freyman et al. 1998) suggest that the site of such a weighting mechanism would reside in auditory nuclei necessary for the processing of directional

information such as the anteroventral cochlear nucleus (AVCN) or nuclei of the superior olivary complex (SOC). There is also evidence that the monaural auditory pathway is involved in the precedence effect. Listeners with sensorineural hearing loss originating in the cochlea, due to hearing damage or aging, show much reduced echo thresholds and localization dominance compared with normal hearing listeners (Goverts et al. 2000 & 2002).

The psychophysical evidence suggests that both monaural and binaural auditory processing contribute to the phenomena of fusion and localization dominance and binaural processing is necessary to account for specific observations.

1.2.1 Neural correlates of the precedence effect

In the search for neural correlates and mechanisms of the precedence effect, recordings of single-neuron activity have been made from monaural and binaural auditory nuclei of the **cat** (Yin, 1994; Parham et al. 1996; Litovsky et al. 1997; Litovsky, 1998; Litovsky & Yin, 1998; Mickey & Middlebrooks, 2000; Reale & Brugge, 2000; Mickey & Middlebrooks, 2001; Litovsky & Delgutte, 2002; Tollin & Yin, 2003), **bat** (Liu et al. 1996; Yang & Pollack, 1998; Burger & Pollack, 2001; Klug et al. 2002; Bauer et al., 2002; Zhou & Jen, 2003), **chinchilla** (Wickesberg, 1996; Backoff et al. 1997; Recio et al. 1997), **rabbit** (Fitzpatrick et al. 1995; Fitzpatrick et al. 1999), **rat** (Kelly, 1974; Kelly et al. 1998; Kelly & Kid, 2000), and **owl** (Takahashi & Keller, 1994; Keller & Takahashi, 1996; Spitzer et al. 2004). Typically, binaural click pairs have been used to simulate a direct sound and a single reflection (Figure 1.1.2) although a limited number of studies have also employed longer duration stimuli.

Neurons in all auditory nuclei of the mammalian and avian auditory systems respond best to the leading sound, and show a reduced response to the lagging sound. Neural suppression of the lagging sound provides a physiological mechanism for temporal weighting favouring the leading sound, and therefore emphasising auditory cues present in the leading sound relative to the lagging sound. Neural suppression has generally been quantified by expressing the number of action potentials evoked by the lagging sound as a percentage of the number evoked by the leading sound (Yin, 1994; Fitzpatrick et al. 1995; Parham et al. 1996; Litovsky & Yin, 1998a; Fitzpatrick et al.

1999). When expressed as a function of the lead-lag interval (LLI) this metric describes the time course of the recovery of the neurons response to the lagging sound. Recovery functions have been used to correlate neural activity with behavioural measures of the precedence effect. When recovery times are averaged across neurons, the time at which the response to the lagging click reaches 50% of that to the leading click best correlates with psychophysical measure of precedence effect sound (Yin, 1994; Fitzpatrick et al. 1995; Litovsky & Yin, 1998a; Fitzpatrick et al. 1999).

In monaural auditory nuclei, the recovery of neural responses to lagging clicks for neurons with centre frequencies (CFs) >1 kHz was found to average 50% for LLIs of 2-3 ms in the auditory nerve (AN) of the cat (Parham et al. 1996; Fitzpatrick et al. 1999) and chinchilla (Wickesberg & Stevens, 1998) and in the cochlear nucleus (CN) of the cat (Fitzpatrick et al. 1997; Parham et al. 1998) and chinchilla (Wickesberg et al. 1996; Backoff et al. 1997). While the average recovery times of neurons between the nuclei were similar, the range of recovery times contributing to the average 50% recovery time was found to be greater in the CN than in the AN (Parham et al. 1998). Nevertheless, the robust response to lagging clicks observed for LLIs between 2 and 3 ms in the majority of neurons found in the AN fibres (ANFs) and CN neurons correlates with the echo threshold measured in human listeners presented with monaural click pairs (Zurek, 1979). Parham et al. (1998) suggest that the broader range of recovery times observed for neurons in the AVCN relative to those observed in ANFs may reflect their contribution to different aspects of the precedence effect; neurons with longer recovery times being more compatible with binaural aspects of the precedence effect where echo threshold is increased, and those with shorter recovery times contributing to monaural precedence effects and aspects of lagging sounds that are not suppressed.

The recovery functions in the AN and CN were shown to depend on the intensity of the click pairs, with increases in intensity resulting in increases in suppression of responses to the lagging click (Parham et al. 1996; Wickesberg et al. 1996; Backoff et al. 1997; Wickesberg & Stevens, 1998). This would be expected from psychophysical data describing increased echo thresholds in human listeners for stimuli of higher intensities (Goverts et al. 2000; Saberi et al. 2004). Additionally, greater suppression was observed for greater numbers of clicks in the click train (Wickesberg & Stevens, 1998).

Such an effect could help explain the build up of the precedence effect under binaural listening conditions where human listeners experience fusion of leading and lagging binaural click pairs only after repeated presentation (Clifton, 1987; Clifton et al. 1994; Clifton, et al. 2002).

Two of the studies above also describe a reduction of the responses to the leading click due to the lagging click. It was necessary to take this backward masking effect into account when calculating recovery functions in both the AN of the cat (Parham et al. 1996) and the CN of the chinchilla (Wickesberg & Stevens, 1998). Furthermore, Wickesberg and Stevens (1998) report a decrease in the synchronization of spike times to the leading and lagging clicks with decreasing LLIs. Backward masking effects and de-synchronization occurring during fusion may have implications for binaural processing where the intervals between leading and lagging clicks differ between the left and right ear, as is the case when the ITD of leading binaural click differs to that of the lagging click. Such changes could alter interaural difference cues represented in neural output from those present in the stimulus (described in more detail in section 1.2.3; Hartung & Trahiotis, 2001).

In binaural auditory nuclei the average time of neural suppression of responses to the lagging click of binaural click pairs is extended relative to those observed in monaural nuclei. This is consistent with echo thresholds observed in psychophysical experiments when using binaural as opposed to monaural stimuli. The LLI at which neural responses to the lagging click of binaural click pairs recovers to 50% averaged across neurons has been reported in the range 20-35 ms for neurons located in the IC of the cat (Yin, 1994; Litovsky, 1998; Litovsky & Yin, 1998a). This range however appears to have been affected by the use of barbiturate anaesthetic as measurements in the un-anaesthetized cat have since been reported at an average LLI of 10 ms (Tollin et al. 2004). This finding is more comparable to the average 50% recovery at a LLI of 12 ms reported for recordings from neurons located in the IC of un-anaesthetized rabbits (Fitzpatrick et al. 1995; Fitzpatrick et al. 1999). Furthermore behavioural measurements of echo threshold in the cat are reported to be ~10 ms (Tollin & Yin, 2003; Tollin et al. 2004) providing validation for the use of the 50% recovery metric as a correlate of behaviour.

As is the case for neurons located in the AN and CN, the recovery of responsiveness to the lagging click in neurons in the IC of the cat has been shown to be mediated by stimulus duration and intensity (Litovsky, 1998; Litovsky & Yin, 1998a; Litovsky & Yin, 1998b; Fitzpatrick et al. 1999). Additionally, the relative levels of binaural click pairs has also been shown to mediate neural suppression with relatively louder leading clicks being more suppressive for IC neurons in the cat (Yin, 1994).

For neurons located in the auditory cortex, responses to leading and lagging sound pairs are similar to those described for neurons located in the IC (Fitzpatrick et al. 1999; Reale & Brugge, 2000; Mickey & Middlebrooks, 2001, 2005). The major difference between the two nuclei is an increase in the time course of recovery of responses to lagging sounds. Fitzpatrick et al. (1999) report an increase of ~13 ms in the 50% recovery of neural responses to the lagging sound from the IC to the auditory cortex of the un-anaesthetised rabbit. Similarly, Mickey and Middlebrooks (2001) report 100% recovery times of up to 400 ms in auditory cortical neurons examined in the anaesthetised cat and 200 ms in the un-anaesthetised cat (Mickey & Middlebrooks, 2005), a value reported as having a maximum of 100 ms in IC neurons examined in the anaesthetised cat (Yin, 1994).

Neurons in binaural nuclei exhibit correlates of the precedence that cannot be accounted for by the responses of monaural neurons. Correlates of summing localization have been demonstrated in neurons located in the IC (Yin, 1994) and auditory cortex (Reale & Brugge, 2000; Mickey & Middlebrooks, 2001) of the cat and in neurons located in the IC of the barn owl (Takahashi & Keller, 1994; Keller & Takahashi, 1996; Spitzer et al. 2004). Responses of single neurons in these nuclei to leading and lagging sounds separated by 1ms are consistent with a sound delivered from a position between the leading and lagging sound. This displays integration of binaural localization cues present in the leading and lagging sounds into a response that represents only a single sound. Several studies have also shown the strength of neural suppression to be dependent on the location of the leading sound in the IC of the cat (Yin, 1994; Litovsky and Yin, 1998a; Litovsky and Yin, 1998b; Tollin et al. 2004) and bat (Burger & Pollack, 2001; Zhou & Jen, 2003). This suggests a binaural contribution to neural suppression that cannot be accounted for by monaural neural suppression. Furthermore, neural suppression in a limited number of IC neurons in the cat (Yin,

1994; Litovsky & Yin 1998b) and rabbit (Fitzpatrick et al. 1995) has been shown to occur when the leading sound does not evoke a response. This appears to rule out discharge history effects that may account for suppression in the auditory nerve, and suppression mediated by glycinergic inhibition seen in the CN (Wickesberg, 1996; Backoff et al. 1997) as potential mechanisms.

The discovery that neural responses to the lagging click can remain suppressed whether or not the neuron responds to the leading click is essential in temporal weighting models of localization dominance that suggest neural suppression of lagging responses. Consider the responses of two neurons that represent the entire population of cells used for sound localization. The first neuron responds only when a sound is presented with the ITD of the leading sound, the second neuron responds only when sound is presented with the ITD present in the lagging sound. For the ITD of the leading click to be weighted more than that of the lagging click, as is the case during localization dominance, the neuron tuned to the ITD of the lagging click must be suppressed by the leading click which in that neuron does not evoke a response. If this were not the case, neural responses would be equally robust for the lead and the lag click when considered over the population of neurons ruling out any dominance of the leading click.

Findings from electrophysiological recordings of neurons located in binaural auditory nuclei suggest that the neural suppression observed in binaural nuclei is essentially different to that seen in monaural auditory nuclei, and that it is not necessarily linked to neural response history. If this were not the case, the factors reported to increase suppression such as signal intensity and duration, and the temporal proximity of leading and lagging sounds, could be well described by adaptation of responses to ongoing stimuli seen in monaural and binaural auditory nuclei. Whilst a broad range of recovery times of responses to lagging sounds are reported, neurons exist in binaural nuclei that show robust responses to lagging sounds within the time frame necessary to account for behavioural measures of echo threshold. The above findings suggest binaural aspects of stimulus fusion and localization dominance are well described by neural suppression observed in responses of neurons in the IC. However, if the precedence effect were entirely to be the result of auditory processing in binaural nuclei, it would have to be represented by neurons that show suppression due to the leading sound even when they are not responsive to it.

1.2.2 Physiological Mechanisms Underlying Neural Suppression

It has long been suggested that inhibitory neurotransmitters in the auditory system may serve to suppress neural activity subsequent to the arrival of an initial sound. This would account for the observed reduction in responsiveness of neurons located in binaural auditory nuclei to lagging sounds (Yin, 1994; Litovsky & Yin, 1998b; Fitzpatrick at al. 1995; Pollack & Burger, 2002).

Monaural neural suppression can be accounted for by neural adaptation at the level of the AN and by neural adaptation and neural inhibition at the level of the CN. Neurons in all divisions of the CN of the chinchilla exhibited shorter recovery times to lagging clicks when the action of γ-amino butyric acid (GABA) or glycine was blocked with the receptor antagonists bicuculline methiodide and strychnine, respectively (Backoff et al. 1997). Under such circumstances, 100% recovery of responsiveness to the lagging click was achieved at a LLI of 2 ms compared with 2-16 ms in the absence of these antagonists, regardless of which was used. Similar observations were recorded when the action of the dorsal cochlear nucleus was reduced through injection of lidocaine (Wickesberg, 1996) suggesting, at least in part, the inhibition observed in such neurons originates in this division of the CN.

Monaural neural suppression is capable of explaining the suppression of all lagging sounds equally, however the directionally dependent inhibition observed in some binaural neurons (Yin, 1994; Litovsky and Yin, 1998a; Litovsky and Yin, 1998b; Tollin et al. 2004), and the extended time course of that suppression, indicates that inhibition in binaural neurons originates in a binaural nucleus. A number of studies have suggested the dorsal nucleus of lateral lemniscus (DNLL) as the most likely candidate (Yin, 1994; Fitzpatrick et al. 1995; Zhou & Jen, 2003). Neurons of the DNLL display similar sensitivity to ITDs as neurons of the IC, and as such, are capable of providing directionally-dependent inhibitory input.

The DNLL with its largely GABA-ergic output, provides the major inhibitory input to the IC in many species (Kelly & Lee 1997; Chen & Kelly, 1999), with projections terminating in both ipsilateral and contralateral IC as well as the contralateral DNLL. The binaural nature of the DNLL would provide it with the capacity to inhibit

responses in a directionally-dependent manner. Studies of species with specialized high-frequency hearing, such as echo-locating bats, have shown the importance of GABA in processing the temporal patterns of sounds (Pollack & Burger 1998; Klug et al. 2002; Burger & Pollack, 2003); e.g. inhibition from the DNLL to the IC of the moustache bat is thought to suppress information arriving from later sounds, possibly annulling their directional information, and creating *de novo* certain response properties in the IC (Burger & Pollack, 2003). Directionally-dependent inhibition of neural responses in the IC of the big brown bat to the lag click of a binaural click pair was abolished by blocking of $GABA_A$ receptors through iontophoretic application of bicuculline, providing direct evidence for this theory (Zhou & Jen, 2003). Findings from the IC of the rat also indicate GABA-ergic inputs are capable of mediating neural response characteristics to lagging sounds (Kelly & Kid, 2000).

In contrast to bats and rats, species with low-frequency hearing, including humans, rely more on ITD information present in low-frequency sounds when locating fused binaural click pairs (Divenyi, 1992; Yang & Grantham, 1997; Tollin & Henning, 1999; Hartung & Trahiotis, 2001). As such, species such as the bat with high-frequency hearing and highly specialized auditory behaviour may not provide a good model for the human auditory system. Nevertheless, Le Beau et al. (2001) have shown that GABA and glycine shape frequency-selective response of IC neurons in guinea pigs, suggesting that observations made in bats may also apply to mammals with low-frequency hearing. It is also known, however, that GABA-ergic inhibition does not alter the ITD sensitivity of low-frequency IC neurons in the guinea pig (Ingham & McAlpine, 2005) in the manner it has been presumed to do (Fitzpatrick et al. 1997). It remains to be determined whether species with similar hearing capabilities to humans, in particular species with similar low-frequency hearing and which use ITDs for sound localization, utilise inhibitory neurotransmitters in echo-suppression tasks.

Binaural nuclei other than the DNLL also provide inhibitory inputs to the IC, and thus potentially contribute to echo processing. Both the lateral superior olive (LSO) and the ventral nucleus of lateral lemniscus (VNLL), which provide inhibitory (glycinergic & GABA-ergic) input to the ipsilateral IC, are potential origins for echo suppression by means of central inhibition (Fitzpatrick et al. 1995; Riquelme et al. 2001). Projections from the LSO and VNLL terminate primarily in the ventral region of the central

nucleus of the IC (ICc). As such, they provide inhibitory input primarily to neurons with high-CFs (see Appendix B for further detail). It is likely that any contribution these nuclei may have to neural suppression would therefore be limited to the processing of high-frequency components of sound (Oliver, 2000).

1.2.3 Alternative Models of Localization Dominance for Low Frequency Hearing Mammals

An alternate hypothesis to one of neural suppression has been proposed to account for the precedence effect observed when localizing binaural click stimuli in species that rely mainly on low frequency interaural cues to localize sound (Tollin, 1998, Tollin & Henning 1999, Hartung & Trahiotis, 2001). This hypothesis does not rely on binaurally-mediated neural suppression of directional information present in lagging sounds to account for localization dominance. Rather, it argues that interactions occur during cochlear processing of the leading and lagging clicks due to a relatively long response time to the leading click. This interaction results in alterations to the ITD and IID cues conveyed by the leading and lagging clicks, particularly at low frequencies. Because species with well developed low-frequency hearing, such as humans, rely on directional cues present in the low frequency components of sound, their localization judgments would be determined by the altered interaural cues at these frequencies integrated over the duration of the entire stimulus waveform.

Tollin (1998), using a computational model of the basilar membrane and auditory nerve followed by a process of cross correlation, predicted the results of psychophysical data without including inhibitory mechanisms in the model. Tollin's model employed a single band-pass (gamma-tone) filter centred near 750 Hz for each ear to mimic the filtering effects of the basilar membrane. The use of a single filter for each ear, while not physiological realistic, was justified by psychophysical data that suggested the frequency region around 750 Hz dominated listeners' lateralization judgements of clicks (Henning, 1983; Tollin & Henning, 1996). Analysis of energy spectra of the combined lead and lag clicks to each ear was then used to adjust the centre frequency of the filter to that closest to 750 Hz containing the most energy. This allowed for the possibility that there may be little energy around the 750-Hz dominance

region in the summed stimulus waveform, a factor which is dependent on the both the LLI, and the ITD, of the leading and lagging click pairs.

Following gamma-tone filtering, the waveform was half-wave rectified, a stage representing the response of ANFs. Additionally, only deflections exceeding 20% of the maximum deflection were retained in order to mimic monaural phase locking normally observed in ANFs at low frequencies. The output from each modelled ANF was then integrated over 5 ms, and cross-correlated to mimic the process of coincidence detection observed in the MSO, to determine the ITDs represented in the stimulus. The ITDs were weighted using a Gaussian window centred at 0 ms ITD, with a standard deviation of 600 μs. This weighting was performed so that physiologically-relevant ITDs were more 'important' in the determination of lateral location (Tollin & Henning, 1999b). A similar process was conducted on the amplitudes of the left and right waveforms to determine the IID represented by the signal. These differences were converted to ITDs (20μs/dB) and linearly combined with the ITD information in order to arrive at the final estimate of laterality suggested by the model.

The Tollin (1998) model predicted the classical data from the experiments of Wallach et al. (1949). The model successfully accounted for both the impact of the ITD of the lagging click in determining listeners' lateralisation judgments, and the counterintuitive reversals in the magnitude of the lagging click ITD, necessary to centre the fused image when the ITD of the leading click reached large values. The model also predicted increased just noticeable differences (JNDs) for changes in the ITD of the lagging click as a function of the LLI and the ITD of the leading click. Further support for the model was provided by a subsequent study (Tollin and Henning 1999a), which showed that listeners' lateralisation judgments of a leading sound followed by two lagging sounds were based on the interaural phase differences (IPDs) indicated by the summed stimulus spectrum for the entire stimulus (i.e. all three click pairs) in the 750-Hz dominance region. These results suggest that listeners do not suppress information contained in low-frequency components of echoes, but rather that the information is integrated over the duration of the stimuli and the subsequent location of the perceived sound determined by the resulting timing cues of the low frequency components of the sound, particularly around 750 Hz.

More recently Hartung and Trahiotis (2001) arrived at a similar conclusion via a more physiological-realistic modeling procedure that did not require spectral or ITD weighting to predict psychophysical findings. The use of processing steps that describe specific physiological processes also provides insight into the possible mechanisms underlying the processing of paired click stimuli as a single event. Using a computational model comprising physiologically-realistic filtering by the basilar membrane and cochlear hair cells, followed by a process of binaural cross correlation, Hartung and Trahiotis (2001) found that the peripheral processing of binaural clicks arriving within milliseconds of each other produces an internal representation of the ITDs and IIDs that, when summed across frequency, could differ significantly to those present in the stimulus; i.e. before cochlear filtering. Similar to Tollin (1998), Hartung and Trahiotis (2001) demonstrated that their model could be used to predict localization dominance data from several experimental studies without the need for binaurally-mediated inhibition. Neural suppression in this case was limited to that occurring at the level of the AN. In each case, when summed across all frequencies, the peak of activity on the cross correlation surface coincided with listeners' localization judgments.

The cochlear processes mimicked by the modeling procedure that produced alterations to ITD and ILD cues, are best understood by examining them individually. The frequency selectivity of the basilar membrane (BM) was modelled using a gamma-tone filter bank, the bandwidths of which were derived from psychophysical measurements of auditory filters in the human cochlea. Filtering the transients with a gamma-tone filter results in a sinusoidal waveform that decreases in amplitude over time (Figure 1.2.1). The periodicity of the waveform is determined by the CF of the filter, being equivalent to the reciprocal or 1/CF. The duration of the waveform is determined by the bandwidth of the filter. The physical properties of the BM means it behaves in an analogous manner to a bank of gamma-tone filters, responding for relatively long durations toward the low frequency end, and relatively short durations at the high frequency end. As such, the bandwidth of frequencies to which inner hair cells are responsive is narrower for cells located at the low frequency end of the BM, with these cells responding for longer when stimulated by a click than those toward the high frequency end. The consequence of this can be seen in the output of ANFs in response to clicks, which are thought to follow closely BM displacement (Lin & Guinan. Jr., 2000). ANFs exhibit a periodic response to clicks determined by their CF, the duration

of which is also determined by CF and the amplitude of the click. ANFs with CFs around 900 Hz can respond anywhere from 3-8 ms depending on the intensity (42-114 dB) of the click (Lin & Guinan Jr. 2000). The important consequence of this is that the duration of the response to the leading click means the BM will still be responding when the lagging click arrives. Depending on the phase relationship at any particular point on the BM, this will result in either addition or cancellation of the combined waveform. This may result in changes to the relative timing or amplitude of the peaks of the combined waveform i.e. the BM representation of the leading and lagging click, from those conveyed by the stimulus. If an ITD is imposed upon the lagging click that differs from that imposed on the leading click, the phase relationship between the clicks at each ear will differ. Since there is a different time delay between the leading and lagging click at each ear the effect on the peaks in the cochlear waveform that represent the leading and lagging clicks will also differ. Evidence for the influence of this was apparent in the responses of CN neurons to monaural click pairs that showed both backward and forward masking as well as de-synchronisation of spikes times with the stimulus waveform that depended on the LLI (see section 1.2.1; Wickesberg & Stevens, 1998). If one were then to consider combining the activity from the left ear with that from the right ear, the stimulus representation at each particular frequency would be of a different duration, with potentially different ITD and IID cues compared with the physical stimulus.

The next stage of processing in the Hartung and Trahiotis (2001) model is the inner-hair cell (IHC), the purpose of which is to transform the filtered waveform into neural output, as seen in the ANF. The filtered waveform is half-wave rectified, a process analogous to the deflection of the stereocilia of IHCs, which are activated in response to movement of the basilar membrane in only one direction. Membrane permeability, translating to the readiness of the IHC to depolarize, is linked to the displacement of the BM, the function being compressive with greater displacement (more intense sounds). Increases in membrane permeability, in turn, result in the release of neurotransmitter into the synaptic cleft at the base of the IHC. The probability of neurotransmitter release resulting in the production of an action potential is finite, and increases with the concentration of neurotransmitter in the synaptic cleft. The model incorporates recycling of neurotransmitter, which serves to enhance the capabilities of the model to predict neural adaptation in response to continuous sounds. A finite amount of

neurotransmitter is available, but is continuously recycled from the synaptic cleft and reprocessed, becoming available again for release. In addition, fresh neurotransmitter is being produced. This configuration allows the model to respond strongly to signal onset or changes in the level of the signal after which a steady state is achieved determined by the amount of transmitter available for release (Meddis, 1986).

The output of the IHC model has been evaluated using a variety of stimuli and compared with the output of the AN (Meddis, 1986, Meddis, 1988). The adapting nature of the IHC component of the model provides a mechanism for monaural temporal weighting of the leading click. The inclusion of the IHC model described by Hartung and Trahiotis (2001) resulted in more accurate predictions of behavioural findings than those made without inclusion of the IHC model (for a detailed description of the cochlea see Appendix B).

The cross correlogram (cross correlation surface) used in the model is a representation of the coincidence detection theory of binaural coding proposed by Jeffress (1948; see Appendix B, Binaural auditory nuclei). Different cells that code for ITD are represented on a grid, with CF on the ordinate and ITD on the abscissa (Figure 1.2.2). The average ITD represented by each CF band, integrated across different bands represents the peak population response of the neural elements in the model. The output of the cross-correlogram has been used to predict successfully the outcomes of a variety of behavioural studies utilising a range of different stimulus types (Bernstein & Trahiotis, 1996, Trahiotis et al. 2001). With BM filtering and IHC processing incorporated into the model, the arrival of the lagging click while the auditory periphery is still responding to the leading click causes frequency-specific changes to the stimulus representation in each ear. The simulated basilar membrane filtering and IHC processing incorporated into the model means the arrival of the lagging click while the auditory periphery is still responding to the leading click alters differently, in a frequency-specific manner, the stimulus representation in each ear. When integrated over a 30-ms period and cross-correlated, this produces ITDs and IIDs different to those originally conveyed by the sound source. In this way, the ITD and IID of the lag click is incorporated into the combined waveform resulting in the peak of the summed cross-correlation surface being pulled toward the ITD indicated by the lag click (Figure 1.2.1).

The modeling of Hartung and Trahiotis (2001) argues for a purely cochlear contribution to localization dominance for low-frequency hearing animals. Changes to directional cues conveyed by the leading *and* lagging clicks are supposed to occur. As such, the responses of low-frequency neurons to the leading portion of the cochlear waveform in response to binaural click pairs represents directional information conveyed by the leading and lagging click and vice versa. If BM filtering followed by temporal weighting at the level of the IHC and subsequent cross-correlation can explain the responses to precedence stimuli, the requirement of binaural inhibition for mediating echo threshold would be minimized.

Experimental support for the Hartung and Trahiotis (2001) model is evident in recent experiments with hearing impaired subjects. Listeners with sensorineural hearing impairment fail to manifest precedence behaviour to the same extent as normal listeners. The cause of this is attributed to poor temporal coding at the level of the cochlea, a situation analogous to poor signal-to-noise environments for normal listeners (Goverts et al. 2000; Goverts et al., 2002). Such effects would not be predicted by a central inhibitory description of echo suppression, whereas predictions of the Hartung and Trahiotis (2001) model are most accurate with the inclusion of IHC processing indicating the important role IHCs may play in precedence effect. Evidence for such effects in physiological data would provide strong support for the peripheral processing hypothesis.

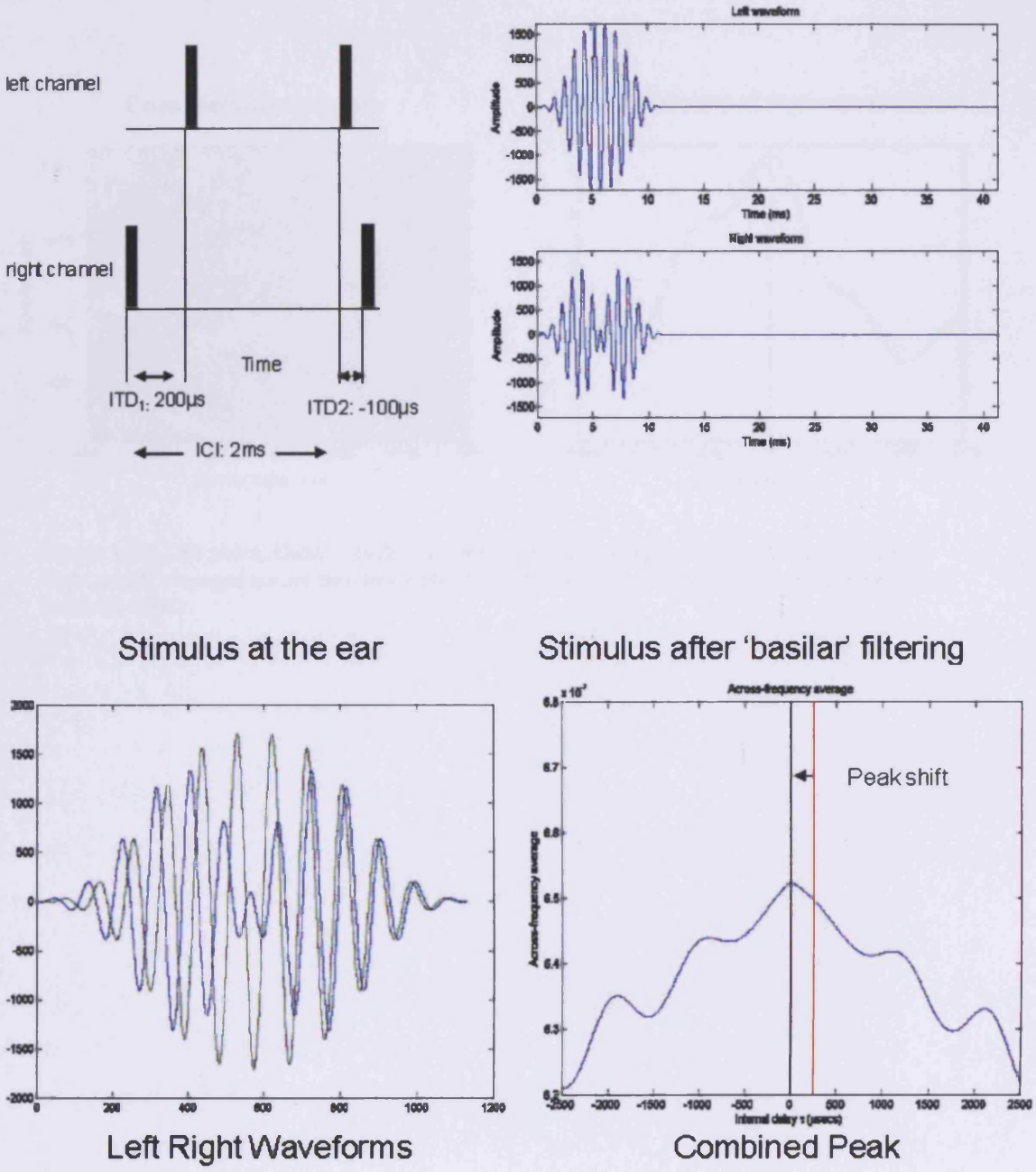


Figure 1.2.1 Stages of cochlear processing resulting in signal transformations as determined by the model of Hartung & Trahiotis (2001). Top left panel is a cartoon of the digital stimulus waveform. Top right panel is stimulus waveform after processing with a 1/3 octave gammatone filter centred at 500 Hz. Bottom left panel indicates the difference between the processing of the left (green) and right (blue) waveforms. Bottom right panel is the across frequency average of the left and right waveforms, when integrated over a 20 ms time window. The red line is the ITD predicted by the stimulus, the black line the shift in ITD after cochlear processing.

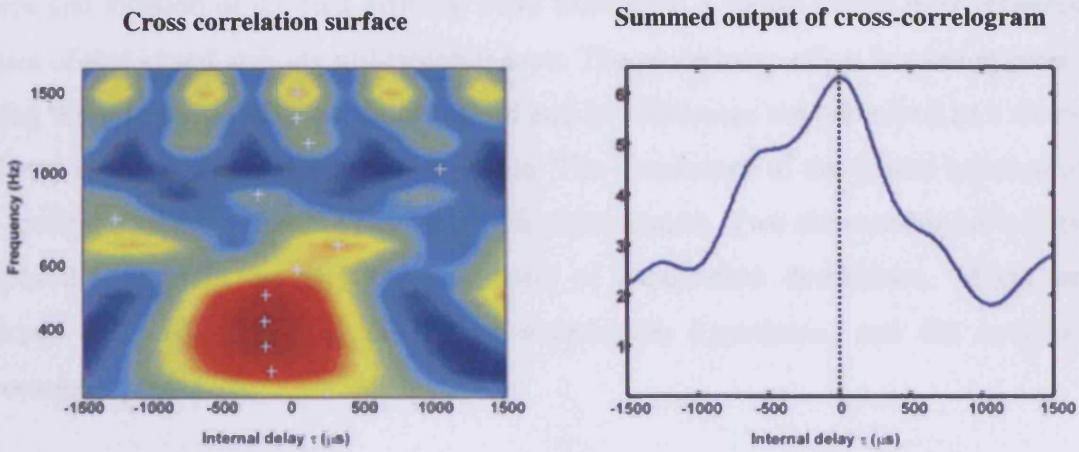


Figure 1.2.2 Left panel; Cross correlogram representation of binaural coincidence detection. Right panel, averaged across frequency peak indicates perceived location of sound (Hartung & Trahiotis 2001).

1.3 Summary of the Physiological Basis of the Precedence Effect

The precedence effect describes the dominance, on auditory perception, of the spatial extent and location of the first arriving wave front from a sound source over reflected copies of that sound arriving milliseconds later. The precedence effect is most apparent during the time over which the direct sound and its reflections are perceived as a single auditory event; a period referred to as fusion. The dominance of the spatial location of the leading sound is referred to as localization dominance. Two alternatives have been proposed to explain the physiological basis of localization dominance, which are referred to in this thesis as the neural suppression hypothesis, and the cochlear processing hypothesis.

1.3.1 The Neural Suppression Hypothesis

Localization dominance can be described as the temporal weighting of directional cues conveyed by the leading sound relative to those conveyed by a later arriving copy of that sound. The greater perceptual weight given to the directional information conveyed by the leading sound explains why listeners hear a sound originating from near the location of the leading sound with a slight bias in localization judgments toward the location of the lagging sound (Wallach et al. 1949). When presented with binaural click pairs representing a direct sound and a single reflection, neurons in the mammalian auditory system respond best to the leading sound and show a reduced response to the lagging sound. Neural suppression of lagging sounds in binaural auditory nuclei offers a physiological mechanism for temporal weighting of directional cues present in leading sounds. Due to the more robust neural response to the leading click directional cues conveyed by the leading sound are represented in such neurons with a greater number of spikes than those conveyed by the lagging sound. Therefore, the combined neural activity in response to the binaural click pairs is weighted in favour of the leading click and, as such, so is the neural representation of directional information. Recent evidence has shown azimuthally-dependent neural suppression of lagging responses in the IC of the big brown bat is mediated by GABA (Zhou & Jen, 2003). It has been also been shown in the Mexican free-tailed bat that inactivation of the DNLL allows neurones of the IC to respond to later parts of sound that previously were suppressed (Bauer et al. 2002). Therefore, in the bat, a species with a highly specialized

auditory system that actively uses echoes to locate prey, it appears that GABA-ergic inhibition from the DNLL suppresses the neural responses of IC neurons to lagging sounds. It has been suggested that a similar inhibitory circuit operates in mammals without specialised hearing. An alternative proposal claims that glycinergic inhibition originating in the LSO may fulfil the same suppressive function proposed for the DNLL (Fitzpatrick, 1995). However, the origins of neural suppression in binaural nuclei remain to be determined in non-specialised species that rely primarily on directional information conveyed by low frequency sound.

1.3.2 The Cochlear Processing Hypothesis

Computational modeling of the auditory system of low-frequency hearing species predicts that binaural click pair stimuli interact in the cochlea resulting in alterations to the binaural directional cues originally conveyed by the leading and lagging clicks of the stimulus (Tollin, 1998; Hartung & Trahiotis 2001). The resulting ITD and IID cues conveyed by the altered internal representation of the leading *and* lagging click explain the behaviour of listeners localising transient stimuli rather than a weighting of the directional cues conveyed by the leading click alone due to neural suppression of responses to the lagging click (Tollin & Henning 1998; Hartung & Trahiotis 2001).

When the cochlea is stimulated with a transient stimulus, such as a click, it resonates. The low-frequency response of the cochlea is of a longer duration than the high-frequency response. When the duration of the cochlea's response to the leading click exceeds the interval between the clicks there is a possibility that interference from the still responding cochlea may alter the cochlea's response to each click. Such interactions would depend on the phase relationship between the cochlea's ongoing response to the leading click and the arrival of the lagging click, but could result in degrees of either enhancement or suppression of the response to either click. The directional information conveyed by the leading and lagging click of a binaural click pair depends on the differences between the waveform at each ear. As such, the interval between the cochlear representation of the leading and lagging click in one ear as well as their relative amplitudes must remain the same as those present in the stimulus in order to convey the same binaural cues. When there are different intervals between the clicks in each cochlea i.e. when the interaural time difference (ITD) of the leading and

lagging clicks differ, the interactions in each cochlea (monaural channel), be they suppressive or enhancive, would be different in each ear. As such, the binaural directional information would be different from that present in the stimulus when represented in binaural auditory neurons. Localization dominance in this conceptualization is the result of low-frequency neurons representing *different* directional cues to those conveyed by the stimulus i.e. high frequency neurons represent the directional cues present in the stimulus whereas low-frequency neurons contain altered directional cues in their representation of *both* the leading and lagging clicks. The combined output of low and high-BF neurons in response to the *leading* click would therefore indicate a direction other than that conveyed by the leading click and likewise the combined output of low and high BF neurons in response to the *lagging* click would indicate a direction other than that actually conveyed by the lagging click. When the responses of the population of high and low-BF neurons to the leading *and* lagging clicks are considered the directional information conveyed by their output would indicate a direction near to that of the leading click but biased in the direction indicated by the lagging click. This theory of localization dominance suggests that the role of binaurally-mediated neural suppression is not important for localization dominance in species with low-frequency hearing. In such species, weighting of the responses to the leading click would not equate to a weighting of the directional cues conveyed by the leading click, but a weighting of the directional cues conveyed by the altered neural representation of the directional cues present in the leading click. Considering that the responses of auditory neurons to the lagging click of a binaural click pair are suppressed for times over which localization dominance operates, it would be necessary for responses to the leading click to be biased in the direction of the lagging click in order to account for its influence on listeners' localization judgments of binaural click pairs.

The models of Tollin and Henning 1998, and Hartung and Trahiotis (2001) were capable of predicting the localization judgements of listeners, suggesting that localization dominance is at least partially determined by cochlear processing. Further evidence for a role of the cochlea has been provided by studies indicating that listeners with cochlear deficits do not experience localization dominance to the same extent as listeners with healthy cochleae (Goverts, 2000; Goverts et al. 2002). However, direct evidence that cochlea processing contributes to the responses of neurons that represent

directional cues in the auditory systems of species with low-frequency hearing is yet to be discovered.

1.4 Aims and Hypotheses

The two primary aims of this thesis are 1) to determine whether there is evidence for a neural representation of directional information in low-frequency neurons that differs from that conveyed by the stimulus in a manner consistent with the cochlear processing model proposed by Hartung and Trahiotis (2001) and 2) to determine whether the suppressive effect of the leading click on neural responses to the lagging click is due to the inhibitory actions of either GABA or glycine in IC neurons of a low-frequency hearing animal. Additionally, characterisation of the responses of low-frequency, ITD-sensitive neurons to single binaural clicks and binaural click pairs will also be undertaken for comparison with the responses of neurons reported in previous studies.

Recordings will be made from single neurons in the IC of the guinea pig in response to single binaural clicks and binaural click pairs. Single binaural clicks and the leading click of the binaural click pair will be presented at each neurons best ITD. The ITD of the lagging click, the lead-lag interval (LLI), and the intensity of the clicks will be manipulated. To assess the role of inhibitory neurotransmitters in neural suppression of responses to the lagging click of a binaural click pair responses will also be recorded during iontophoresis of the $GABA_A$ receptor antagonist gabazine and the glycine receptor antagonist strychnine.

Characterisation of Responses of Low-Frequency Neurons to Binaural Clicks

A systematic study of low-frequency neurons in the IC to paired click stimuli will be undertaken to characterise the responses of this previously neglected sub-population of neurons. Changes in the magnitude of the response to a single binaural click will also be assessed in relation to the intensity and the ITD of the click.

Cochlea Processing Hypotheses

1) It is hypothesised that for binaural click pairs delivered with the same ITD, the lagging click of a binaural click pair will either suppress or enhance a neuron's responses to the leading click depending on the LLI of the stimulus. It is further

hypothesised that the enhancive or suppressive effect of the lagging click will be greater for low-BF neurons than for high-BF neurons.

2) It is hypothesised that for a particular LLI a neuron's response to the leading click of a binaural click pair will be either suppressed or enhanced depending on the ITD of the lagging click.
3) It is hypothesised that any observed suppressive or enhancive effects of the lagging click of a binaural click pair on neural response to the leading click will be related to the intensity of the stimuli. It is expected that more intense stimuli will result in a greater influence of the lagging click.

Neural Suppression Hypotheses

4) It is hypothesised that blocking GABA-ergic inhibition with the $GABA_A$ receptor antagonist gabazine will result in a decrease in the suppressive effect of the leading click of a binaural click pair on neural responses to the lagging click.
5) It is hypothesised that blocking glycinergic inhibition with the glycine receptor antagonist strychnine will result in a decrease in the suppressive effect of the leading click of a binaural click pair on neural responses to the lagging click.

2.0 Methods

2.1 Surgical Procedure

All experiments were conducted in accordance with the Animals (Scientific Procedures) Act 1986 of Great Britain and Northern Ireland. Single neuron recordings were made from the central nucleus of the right inferior colliculus (IC) of pigmented guinea pigs (*cavia porcellus*). All animals were adults, in the weight range 300-500g at the time recordings were made. Anaesthesia was induced with an intraperitoneal (i.p.) injection of urethane (1.3 g/kg in 25% solution of 0.9% NaCl ;Sigma-Aldrich, Poole, U.K.) and an intramuscular injection (i.m.) of fentanyl–fluanisone (0.105 mg/kg of fentanyl citrate and 3.333 mg/kg of fluanisone in sterile solution; 0.3ml/kg of Hypnorm, Janssen-Cilag Ltd., High Wycome, U.K.). Areflexia was monitored by testing the paw withdrawal reflex. Anaesthesia was maintained with additional doses of Hypnorm (0.3ml/kg) and, when necessary, urethane (0.4 g/kg). A subcutaneous (s.c.) injection of atropine sulphate (0.2 mg/kg; 0.3ml/kg of Atropine Sulphate; Animalcare Ltd., York, U.K) was administered prior to surgery to reduce bronchial secretions. A local anaesthetic of lidocaine hydrochloride (2%; Martindale Pharmaceuticals, Romford, U.K) was injected subcutaneously at all surgical sites. The left and right tragi were transacted with scissors to improve access to the ear canal and allow clear visualisation of the tympanic membrane. The ear canal was inspected with an operating microscope and any obstructions to the tympanic membrane removed with splinter forceps. A tracheotomy was performed and the trachea cannulated with a length of flexible Teflon tube tied firmly in place with surgical thread. Mechanical ventilation (Harvard Apparatus Ltd., Kent, England) with air was provided if respiration became difficult. Experiments were performed in a sound attenuating chamber (IAC, Winchester, UK) with animals positioned in a stereotaxic frame. Hollow ear speculae (modified from model 1730, David Kopf Instruments, CA) were inserted firmly into each ear canal, whilst ensuring a clear path to the tympanic membrane. The ear speculae served to fix an animal's head in place and also as a sound conduit for the speaker system. The core temperature of the animal was monitored with a rectal thermometer and maintained a 37°C with a heating blanket placed underneath the animal (Harvard Apparatus Ltd.). The head was made approximately level in the

rostro-caudal plane and fixed in place by a bite bar inserted into the mouth. The skin overlying the skull was dissected away and the underlying temporalis muscles and periosteum reflected to expose the parietal and occipital bones of the skull and the bullae. The angle of the head in the rostro-caudal plane was determined at two positions, separated by 1 cm, on the dorsal surface of the skull with the aid of a two-pronged attachment fixed to a micromanipulator. The angle of the head was adjusted such that the points of both prongs just touched the surface of the skull. A small hole was made in each bulla with the point of a scalpel and a polyurethane tube (inner diameter, 0.67 mm; outer diameter, 0.91 mm; length, 30 cm) with high acoustic impedance inserted and sealed with petroleum jelly (Vaseline) to allow equalisation of the air pressure in the middle ear. A craniotomy was performed extending 2–3 mm rostral and caudal of the interaural axis and 1–4 mm lateral from the midline on the right side by thinning the skull with a grinding bit attached to a small electric drill and enlarging the hole with rongeurs. The *dura mater* covering the exposed cortex was lifted with splinter forceps and cut away with a sharp hypodermic needle. The hole in the skull was filled with agar (2%) or with Vaseline to maintain the integrity of the cortex. On completion of an experiment, a lethal injection of sodium pentobarbitone (0.27 g/kg; 6.6ml/kg Pentoject; Animalcare Ltd.; 60mg/ml) was administered.

2.2 Electrodes

Extracellular single-neuron action potentials were recorded with glass-coated tungsten microelectrodes with tip exposures of 5-10 μm. Tungsten wire was etched to a point in a bath of potassium nitrate and insulated with molten glass and the tip of the tungsten exposed according to the procedure outlined by Merrill and Ainsworth (1971) on a microelectrode manufacturing workstation of the design described by Bullock et al. (1988).

Where experiments required iontophoresis of drugs, tungsten electrodes were glued to multi-barrel glass pipettes. Five-barrel pipettes were pulled on an electrode puller (Narishige; model number 1754-6; Tokyo, Japan) from borosilicate fused-glass capillaries (outer diameter, 1.2 mm; inner diameter, 0.68 mm) containing a glass fibre (WPI, Stevenage, U.K; product code: 5B120-F-4). The glass tip was broken back by inserting it into a bead of molten glass with a micromanipulator and allowing the glass to cool. Upon cooling, the tip broke away remaining in the bead and the resulting open tip diameters of the pipettes were reliably in the range 10-15 μm. Using this technique it was rare to encounter blockages of the capillaries and excellent control in determining the tip diameter was possible. The tungsten recording electrode was then glued with cyano-acrylate (Super Glue Gel) to the glass capillaries within 10μm of their tip. The multi-barrel pipette was fixed to the slide holder of a fixed stage microscope with a clamp while the tungsten electrode was held in a micromanipulator (Figure 2.2.1, photos 1 & 1a) and positioned such that the tips of the two electrodes faced the same direction and were angled toward each other. The tungsten electrode was then advanced toward the tip of the glass pipette until the tips were within 10μm. The tungsten electrode was then moved in the direction of the pipette until they were pressing firmly against each other (Figure 2.2.1, photo 2). The pipette was positioned such that the tungsten electrode seated itself in a groove between two of the glass capillaries which ensured horizontal and vertical alignment of the electrodes (Figure 2.2.1, Photos 4a & 4b). The proximity of the tips was rechecked and adjusted if necessary. The tungsten electrode was then moved outward from the pipette and a bead of Super Glue applied to the pipette with the end of a pair of splinter forceps within ~10μm of the tip. The tungsten electrode was then wound toward the pipette until the two electrodes were pressed firmly together, and the glue was allowed to dry (Figure

2.2.1, photo 3). Heat-shrink tubing was placed over the electrode shaft to prevent separation and more Super Glue applied if necessary (Figure 2.2.1, photo 4).

Prior to the experiment, one barrel of the pipette was filled with sodium chloride (0.5 M; pH 3.5), and used to balance the ejection current. Three of the remaining barrels were filled with either the $GABA_A$ receptor antagonist SR 95531 hydrobromide (25 mM, in distilled water, pH 3-3.5; Gabazine; Tocris; Avonmouth, U.K) or the glycine receptor antagonist strychnine (10 mM, in distilled water, pH 3-3.5; Sigma-Aldrich, product number SO 532). Silver chloride wires were inserted into each barrel of the pipette to pass retention and ejection currents from a NeuroPhore BH-2 iontophoresis system with IP2 current pumps (Digitimer; Hertfordshire, U.K). The resistances of the drug barrels were checked regularly to determine whether they were blocked. If blockages persisted the electrode was discarded. When not in use, gabazine and strychnine required negative retention currents of 10 to 20 nA to prevent unwanted release from the pipette. Positive ejection currents were used to eject the drugs with the current depending on the particular resistance of the drug barrel (~30 nA). Neural recordings in response to the stimulus were made approximately every two minutes until the neurons spike count doubled, after which the ejection current was terminated. Recordings continued until the neurons spike count returned to the control value.

Electrodes were mounted on a piezo-stepped microdrive attached to a micromanipulator (Burleigh Instruments, New York, USA). The electrode was positioned according to sterotaxic coordinates (Medvedev, 1977; Palmer et al., 1990) ~2 mm above the IC and advanced ventrally using a remote control located outside the sound attenuating chamber. Electrical signals from the electrodes were conducted via a low impedance head stage to a preamplifier (Medusa RA16PA, Tucker Davis Technologies System 3, Gainesville, FL., U.S.A) and then via fibre optic cable to an RA16 signal processor (Medusa RA16, Tucker Davis Technologies System 3) where the signals were amplified (×1000 gain), digitized (25 kHz, sampling rate) and filtered (Butterworth high pass filter with a 300 Hz corner frequency; Butterworth low pass filter with a 6 kHz corner frequency).

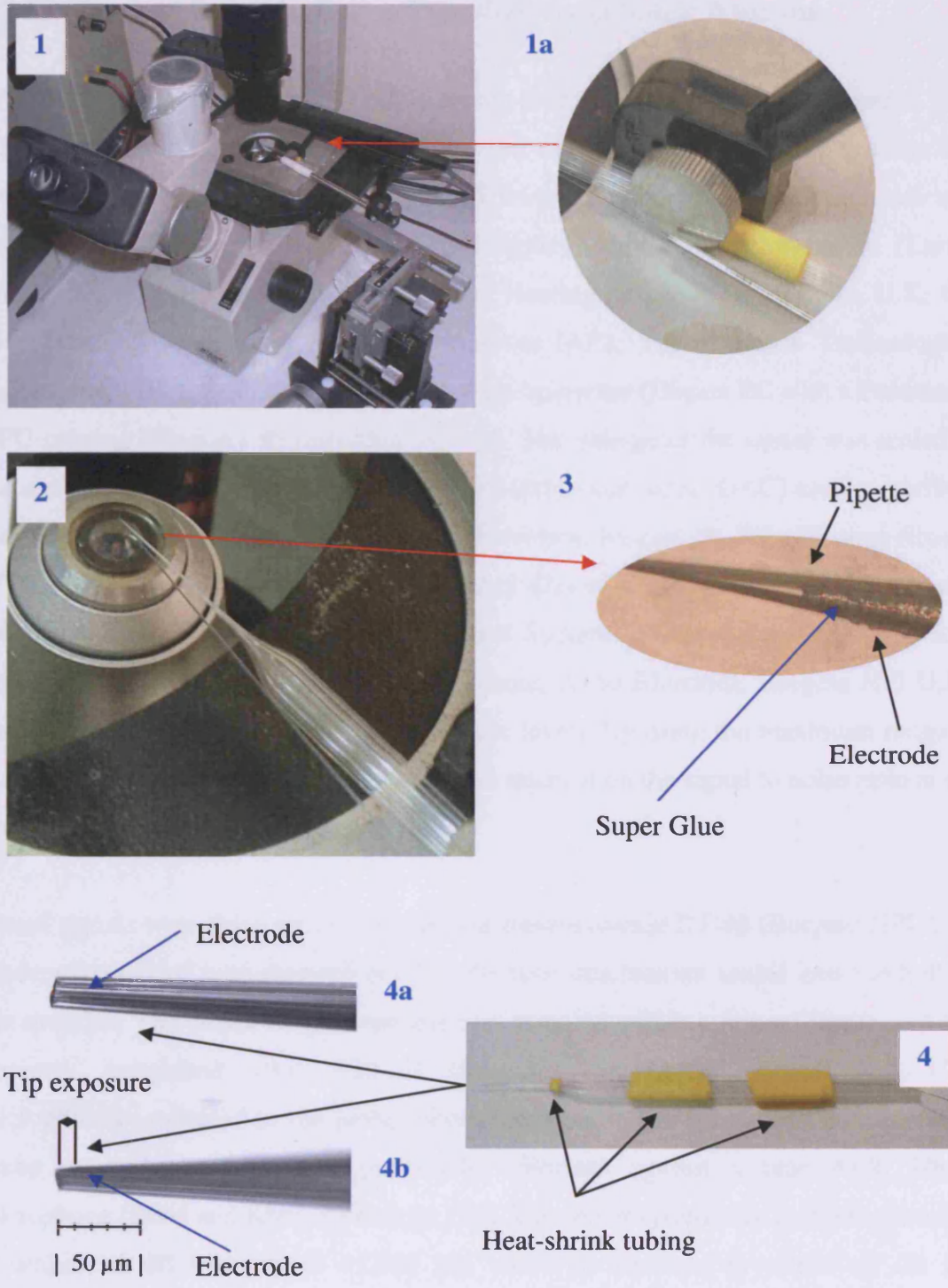


Figure 2.2.1 Iontophoresis electrode manufacture. 1. Microscope and micromanipulator used to position 5 barrel pipette and tungsten electrode. 1a. Close up of holders for pipette and electrode. 2. Super Glue applied to pipette and electrode wound into position. 3. Close up of pipette and electrode with Super Glue. 4. Finished iontophoresis electrode with heat-shrink reinforcing. 4a. Iontophoresis electrode tip in vertical plane, tungsten electrode is positioned behind pipette. 4b. Iontophoresis electrode in horizontal plane. Tip exposure for the tungsten electrode shown. Scale indicated on bottom with 10 µm divisions.

2.3 Finding and Isolating Action Potentials from Single Neurons

Whilst advancing the electrode, a diotic search tone, 50 ms in duration and shaped with a 5-ms rise and fall time, was presented at a rate of 5 Hz in order to locate neurons that responded to auditory stimuli. The desired frequency and intensity of the tone was chosen with a mouse pointer on a visual display using computer software (Trevor Shackleton & Alan Palmer, MRC Institute of Hearing Research, Nottingham, U.K; 100 kHz sampling rate) using an array processor (AP2; Tucker Davis Technologies, Gainesville, FL., U.S.A) mounted in a personal computer (Elonex PC with a Pentium II CPU running Windows 98 operating system). The voltage of the signal was scaled to the maximum range (± 10V) of the digital to analog converter (DAC) used to perform the conversion (DA3-2, Tucker Davis Technologies, System II). Stimuli were filtered (FT6; corner frequency = 40 kHz; Tucker Davis Technologies System II) and attenuated (PA4, Tucker Davis Technologies System II) to the desired level before undergoing fixed amplification (Beyer Dynamic, A150 Blueprint; Burgess Hill U.K.) and further attenuation (60 dB sound pressure level). By using the maximum range of the DAC followed by fixed amplification and attenuation the signal to noise ratio at the speakers was maximised.

Sound signals were delivered to each ear via Beyerdynamic DT-48 (Burgess Hill, UK) loudspeakers fitted with damped brass probe-tube attachments sealed into the hollow ear speculae. The output of each speaker was sampled within a few millimetres of the tympanic membrane using FG3452 (Knowles Electronics, Burgess Hill, UK) microphones connected to the probe tubes embedded in the hollow ear speculae. The probe microphones had been previously calibrated against a type 4136 1/8-in microphone (Brüel and Kjær, Stevenage, UK). The sound systems for each ear were flat to within ±5 dB from 50 to 12,000 Hz, and were matched to within ±5 dB for frequencies below 2000 Hz, and within ±10 dB between 2000 Hz and 12 000 Hz. The maximum output of the system at 1 kHz was 106 dB SPL.

Neural activity from the RA16 was monitored on a Tektronix TDS 210 oscilloscope. On encountering an increase in neural activity in response to the search stimulus, single neuron activity was distinguished from background activity according to two methods. Where Tucker Davis Technologies (TDT) System II hardware was used, action

potentials (spikes) from a single neuron were isolated by adjusting the trigger level of an SD1 spike discriminator (TDT System II) above the level of the background neural activity. Spikes that were greater in amplitude than the defined trigger level were time stamped with 1μs accuracy using an EC1 event timer (TDT System II) and recorded to a file stored on computer. The amplitude and shape of the discriminated spikes was monitored on a second oscilloscope (Tektronix, TDS 210) to ensure isolation of a single spike. Where TDT System 3 hardware was used, the neural signal from the RA16 was monitored and discriminated on the computer in the manner described, using TDT Brainware spike acquisition software. Any recorded spikes (determined by the trigger level) could be further discriminated after recording on the basis of several action potential shape parameters recorded with the spike time (3-μs accuracy). As such the trigger level could be set closer to the level of the background activity and spikes from multiple neurons recorded simultaneously and discriminated after recording.

2.3.1 Location of neurons within the IC

Following isolation of a neuron, the frequency that elicited a response at the lowest sound intensity (best frequency, BF) was determined audio visually using the search tone. This procedure was repeated at several different electrode penetration depths and the estimated BFs and thresholds recorded. The rostra-caudal and medio-lateral boundries of the IC were mapped by repeating this procedure at different sterotaxic coordinates and the response characteristics of the neurons encountered along each electrode track noted with the coordinates.

Neurons located in the central nucleus of the IC (ICc) were distinguished from those located in the pericentral nucleus (ICp) and the external nucleus (ICx) of the IC by the tonotopic organisation of the neurons and their stimulus filtering characteristics. In contrast to neurons located in the ICp and ICx the BFs of neurons in ICc of the cat exhibit a tonotopic gradient from low to high in a dorsal to ventral direction (Rose et al. 1963; Aitkin, et al., 1974; Aitkin & Moore, 1974; Semple & Aitkin, 1979). Neurons located in the ICc of the cat and guinea pig also respond to a narrower range of sound frequencies and have lower response thresholds than neurons located in the ICp and ICx (Aitkin et al. 1974; Syka, et al. 2000). Additionally, neurons of the ICp and ICx

habituate to a stimulus responding for only the first few presentations a feature not seen in neurons located in the ICc (Aitkin et al. 1974). Frequency selectivity and threshold at best frequency were used to confirm the location of the neuron in the IC and further experimental stimuli presented.

2.5 Stimulus Design and Analysis

For all neurons frequency-versus-intensity response areas, interaural time difference functions (ITD functions) and rate versus intensity functions (rate-intensity functions) were recorded prior to recording responses to experimental stimulus configurations. All stimuli were presented in a pseudo randomised order without replacement.

2.5.1 Frequency Versus Intensity Response Areas

Frequency-versus-intensity response areas were recorded by presenting 50-ms tones shaped with a 5-ms rise and fall time that varied in frequency and intensity. The frequencies presented ranged from 4 octaves below the estimated BF to 2 octaves above the estimated BF. The intensity of the tone was varied from 90 dB attenuation to 10 dB attenuation, relative to the maximum output to the system (110 dB SPL for a 1-kHz tone), in 5dB increments. Frequency-versus-intensity responses areas were used to confirm the neurons BF and as an estimate the filter bandwidths of the monaural inputs to the cell.

2.5.2 Binaural Click Stimuli

Binaural clicks were designed using Realtime-Processor Visual Design Studio (RPvds) software (TDT System 3) and generated and filtered using RP2 digital signal processors (50 kHz sampling rate; TDT, System 3). The stimulus intensity for the left and right channels was controlled independently via two PA5 programmable attenuators (TDT System 3) or scaled within the RPvds software. All other aspects of sound delivery were as described for TDT System II hardware (section 2.3). Clicks were created using square pulses of 100-μs duration, filtered by a lowpass filter (Butterworth, with a corner frequency of 40 kHz). A 5-ms delay was incorporated into the stimulus design and a further 1.6-ms delay was introduced to account for the D/A conversion. Therefore the stimulus arrived at the ears 6.6 ms after triggering the DSP. Stimulus triggering and presentation was computer controlled via TDT Brainware software. The peak intensity of clicks measured at the eardrum with 0 dB attenuation measured 95 dB SPL peak (Parham et al. 1998).

2.5.3 Interaural Time Difference Functions

Interaural time differences (ITDs) were imposed on binaural clicks by advancing the timing of one click and delaying the timing of the other click by half the duration of the specified ITD (ms). This was achieved by subtracting half the value of the ITD for the advanced click from a delay of 5 ms and adding half the value of the ITD for the delayed click to a delay of 5 ms. For negative ITDs, the click in the ear contralateral to the recording site (left) was advanced while the click in the ear ipsilateral to the recording site (right) was delayed by an equal amount. For positive ITDs the click in the ipsilateral ear was advanced while the click in the contralateral ear was delayed by an equal amount. For ITD functions, binaural clicks were presented at 40 dB relative to the threshold of the neuron. The average number of spikes evoked by 40 presentations of each ITD was plotted and a neuron's best ITD designated as the ITD that evoked the greatest number of spikes within the range -1.5ms to +1.5 ms (see Figure 2.6.1 Interaural time difference function). For longer ITDs separate responses to each monaural click could be seen in some neurons. Therefore, the selection of best ITDs was restricted to the range -1.5 to + 1.5 ms to ensure responses were not the result of a response to each monaural click. Where binaural click pairs were presented this also ensured that the interval between the leading and lagging click was longer than the ITD. For neurons with ITD functions where there were multiple ITDs that evoked near to maximal discharge rate (panels B, C, and D, Figure 3.1.5, Results Part I), the ITD closest to 0 ms was selected. For neurons that did not respond preferentially to any ITD, 0 ms ITD was selected in place of a best ITD.

2.5.4 Rate Versus Intensity Functions

The number of spikes evoked in response to a binaural click presented at best ITD as a function of the intensity of the click was measured in all neurons. The range of intensities was varied from response threshold at BF to the maximum output of the system in 5 dB steps. Rate-intensity functions were created by plotting the average number of spikes evoked by 40 presentations of each binaural click as a function of the intensity of the click (see Figure 2.6.1 Rate versus Intensity function).

2.5.5 Binaural Click Pair Stimuli

Where pairs of binaural clicks were presented, three parameters were varied; 1) the ITD of the lagging click, 2) the time between the arrival of the leading and lagging click (lead-lag interval, LLI) and 3) the intensity of the leading and lagging clicks. The LLI was defined from the onset of the leading click to the onset of the lagging click (see Figure 2.6.2). Three different stimulus configurations were presented in which the ITD of the leading click was fixed at the best ITD of the neuron. All stimulus configurations included presentation of single binaural clicks presented at the neurons best ITD. Stimuli were presented at a rate of 5 Hz.

For the first stimulus configuration, the LLI was varied from 1-5 ms in 1-ms increments, the ITD of the lagging click was varied from -1.5 - +1.5 ms in 0.1-ms increments and the intensity of the binaural click pair was varied from 5 dB SPL above the neurons threshold to a binaural click presented at best ITD to 95 dB peak SPL, in three steps. The variations in each parameter in this stimulus configuration resulted in 558 different stimulus conditions. As such, the number of repeats of each condition was restricted to 20 and the full data set took approximately 45 minutes to complete. This stimulus configuration was used to assess the effect of backward masking on the neural representation of a leading click in low-BF, ITD-sensitive neurons as a function of the lagging click ITD and the intensity of the binaural click pair.

For the second stimulus configuration, the LLI was varied from 1-10 ms in 1-ms increments, the ITD of the lagging click was presented at the best ITD of the neuron and the intensity of the binaural click pair was varied from the lowest intensity that evoked a response (threshold) to the intensity at which the number of spikes no longer increased with increasing intensity (saturation) in 5 dB increments. This stimulus configuration was used to test the effect of neural suppression on the response to the leading click (backward masking), of neural suppression of the response to the lagging click (forward masking), and the correlation of the neural response with the stimulus waveform as a function of the neurons BF (ranging from low to high).

For the third stimulus configuration, the LLI was varied from 1-20 ms in 2-ms increments, the ITD of the lagging click was presented at the best ITD of the neuron and the intensity of the binaural click pair was set at the intensity that fell halfway

between threshold and saturation as determined from a neurons rate-intensity function. For neurons insensitive to the ITD of binaural clicks, binaural click pairs were delivered diotically (i.e. with an ITD of 0 ms). This stimulus configuration was presented to neurons in which iontophoretic application of inhibitory neurotransmitter receptor antagonists was performed to determine whether neural inhibition due to the action of γ-aminobutyric acid (GABA) or glycine could account for the neural suppression of responses to the lagging click. Binaural click pairs were presented at an intensity that allowed the response to the leading and lagging clicks to increase without the response saturating. In this way it was possible to determine whether release from inhibition led to a selective increase in the response to the lagging click or whether the inhibition suppressed the response to both the leading and lagging clicks. The stimuli were presented prior to, and at regular intervals during application of the receptor antagonists as well as following termination of application. The degree of neural suppression of the response to the lagging binaural click was compared between conditions where inhibition was present (control) when inhibition had been blocked (inhibition blocked) and after the effect of the inhibitory neurotransmitter receptor antagonist had decreased (recovery).

2.5.6 Analysis of Neural Responses Evoked by Binaural Click Pair Stimuli

To analyse neural responses evoked by presentation of binaural click pairs, two discrete time windows of equal duration were used to count the number of spikes evoked by the leading click, and the number of spikes evoked by the lagging click. Responses to the leading and lagging clicks were windowed in one of two ways. For the analysis presented in Part 2 of the results (Chapter 4) the start of the leading click window coincided with the latency of each neurons response to a single binaural click. To determine the response latency of each neuron, the spontaneous neural activity was determined by averaging the number of spikes that occurred in 1-ms time bins for 10 ms prior to stimulus presentation. The first post-stimulus spike time occurring in the first time bin to contain a greater number of spikes than the spontaneous spike count was defined as the response latency. Response latencies were confirmed visually by plotting a dot raster of post-stimulus spike times. The end of the time window used to calculate responses to the leading click was the end of the response to a single binaural click defined as a reduction in spike activity below the neurons spontaneous firing rate.

The start of the lagging click window was the onset of the response to a single binaural click plus the LLI of the stimulus. The duration of the lagging click window was equal to the duration of the leading click window. Any spike times that occurred at the same time as the start of each window and prior to the end of the each time window for each presentation of the stimulus were counted as the response evoked by the corresponding binaural click.

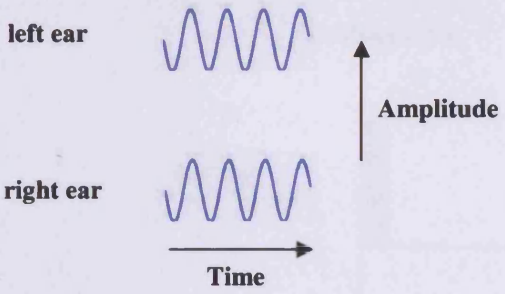
It is apparent that the leading and lagging windows will overlap for LLIs shorter than the duration of the leading click response window i.e. if the response to a single binaural click is longer than the LLI. For such circumstances it is not possible to assign the spikes occurring during the overlapping periods to either the leading or lagging clicks with certainty. One approach has been to sum the number of spikes occurring over the duration of both time windows (the onset of the leading window to the offset of the lagging window) and subtracting the number of spikes in response to a single binaural click to estimate the response to the lagging click. This response is then compared with the neurons response to a single binaural click (Fitzpatrick et al. 1995; Litovsky & Yin, 1998a; Tollin et al. 2004). As discussed in the introduction, this method of estimating the response to the lagging click is most accurate when the neurons response to the leading click is not affected by the lagging click. Recovery functions were calculated for a neurons response to the lagging click by expressing it as a percentage of the neurons response to the leading click where the response windows didn't overlap, and as a percentage of the response to a single binaural click where the response windows did overlap (Fitzpatrick et al. 1995; Litovsky & Yin, 1998a; Tollin et al. 2004). Additionally, recovery functions were calculated only for LLIs where the response windows did not overlap.

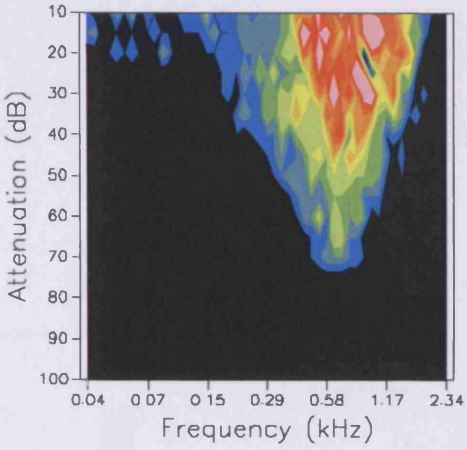
The second definition of the leading and lagging click response windows was utilised in parts three (Chapter 5) and four (Chapter 6) of the results. For the analyses presented in these chapters, the start of the leading click response window was the latency of a neurons response when presented with a single binaural click. This followed the same method used in the first definition described in the first paragraph of this section. The end of the leading click window was the time of arrival of the lagging click rather than the end of the response to a single binaural click as was the case for the previous definition. The lagging click response window began at the time of arrival of the

lagging click relative to the neurons response latency and was of an equivalent duration to the leading click response window. Spikes were assigned to either window if they arrived at the same time as the start of the window and prior to the end of the response window. Where there was overlap of the leading and lagging response windows the spike count obtained from the lagging click window was adjusted by subtracting the number of spikes counted during the overlapping period in response to a single binaural click.

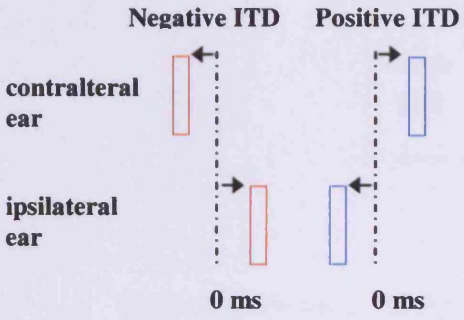
The recovery functions presented in part four of the results were calculated by dividing the spike count from the lagging click window by the spike count derived from the leading click window and multiplying the result by 100 i.e. expressing the lagging click responses as a percentage of the response to the leading click. Using this method, where the response to the leading click may have overlapped that to the lagging click, as indicated by the neurons response to a single binaural click, the recovery functions were calculated on a portion of the neurons response to the leading and lagging clicks. As such, they did not capture the neurons entire response to the leading or lagging click.

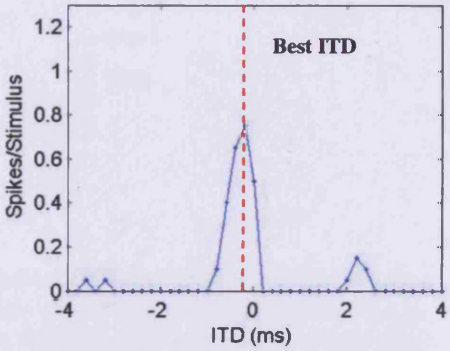
A. Frequency versus intensity Response A



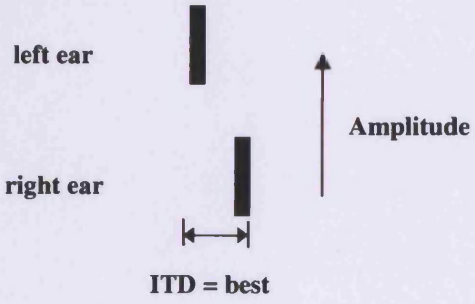


B. Interaural Time Difference Function





C. Rate versus Intensity Function



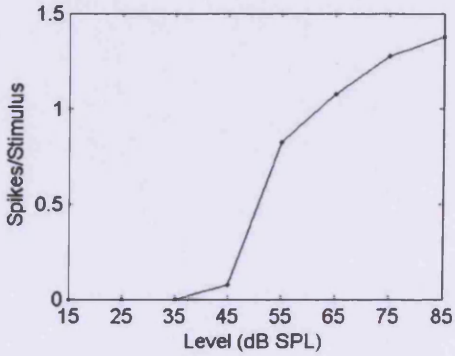


Figure 2.6.1 Three stimuli used to characterise neural responses. Stimuli are shown on the left and typical responses on the right. A. Frequency versus intensity response area; Stimuli; 50 ms diotic tones shaped with 5ms rise and fall times of varying intensity and frequency. Response; the intensity of the tone is plotted on the ordinate and the frequency on the abscissa. Warm colors represent higher firing rates. B. Interaural time difference function; Stimuli; binaural click pairs presented at 40 dB re/ threshold with interaural time differences (ITDs) ranging from – 4 ms to + 4 ms. Response; Number of spikes per stimulus plotted as a function of the ITD of the binaural click. C. Rate verses intensity function. Stimulus; binaural click pair presented at best ITD with intensity varied in 5 dB steps.

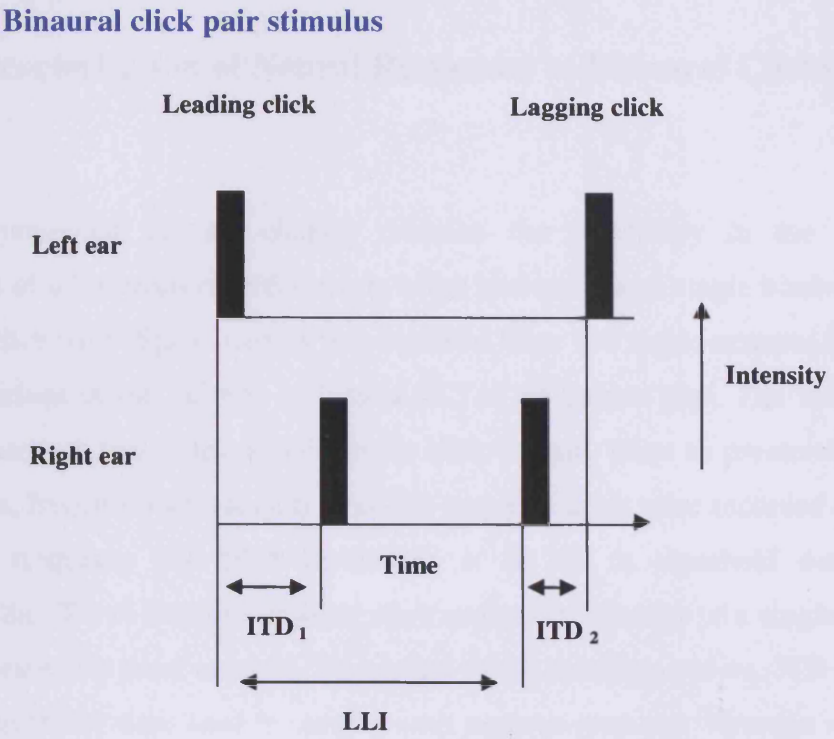


Figure 2.6.2 Binaural click stimulus. The interaural time difference of the leading click (ITD_1) was set at the best ITD for each neuron. The lead-lag interval (LLI), the ITD of the lagging click (ITD_2) and the intensity of both binaural clicks were varied according to the hypothesis under scrutiny.

3.0 Results Part I:

Characterisation of Neural Responses to Binaural Clicks

The results presented in this chapter describe the variability in the response characteristics of all neurons recorded from, when presented with single binaural clicks and binaural click pairs. Spike activity was recorded from 108 single-neurons located in the central nucleus of the inferior colliculus (IC) of 16 guinea pigs. The sample was restricted to neurons that were responsive to click stimuli. Prior to presentation with binaural clicks, frequency-vs.-(sound) intensity response areas were recorded and each neurons best frequency and filter bandwidth at 10 dB re. threshold determined. Sensitivity to the ITD of a single binaural click and to the intensity of a single binaural click were assessed for most neurons. The shapes of the resulting rate-vs.-ITD and rate-vs.-intensity functions were used to classify each neurons response. Neurons were then presented with binaural click pair stimuli and the responses analysed for binaural click pairs delivered with the same ITD and separated by LLIs ranging between 2-10 ms. Responses were characterised according to the temporal patterns observed in each neurons post stimulus spike times in response to the leading click and further classified according to their response to the lagging click.

3.1 Responses to Single Binaural Clicks

3.1.1 Frequency Selectivity

The sample of neurons largely comprised neurons tuned to low frequency sounds (< 2.0 kHz). Figure 3.1.1 shows two frequency-vs.-intensity response areas typical of neurons in the population, one tuned to a low best frequency (left, BF = 585 Hz), and one to a high best frequency (right, BF = 5000 Hz), where BF is defined as the tone frequency that elicited excitation at the lowest sound intensity. Figure 3.1.2 shows the distribution of BFs in the population of neurons, the majority of which had BFs below 2 kHz (73/108).

The 10-dB bandwidth of each neuron was derived from its frequency-vs.-intensity response area by measuring the extent of sound-evoked activation 10 dB above BF threshold. Figure 3.1.3 shows that, with few exceptions, 10-dB bandwidths of neurons with low BFs were narrower than those with high BFs.

3.1.2 Sensitivity to Interaural Time Differences

The discharge rates of nearly two thirds of neurons (67/108; 62 %) were modulated by >30 % by varying the ITD of a binaural click (40 dB re. threshold) over the range -4 ms to +4 ms in 0.2 ms steps. The resulting ITD functions could be categorised as belonging to one of four types (Figure 3.1.4). Panel A in Figure 3.1.4 shows an ITD function from a neuron that responded most vigorously when the ITD of the click was -0.2ms, less vigorously when the ITD was 2.2ms, and less so again when the ITD was -3.6ms. The form of this function, with a prominent "central peak" (defined as the best ITD) and unevenly damped "side peaks", was evident in the ITD functions of half (34/67; 50.8 %) of ITD-sensitive cells (**peak** response type). Panel B of Figure 3.1.4 shows the response of a neuron that responded vigorously when the ITD was -0.2ms and again at 1.6 and -2.0ms, a separation of approximately 1.7ms. This **periodic** response type, where the central peak was flanked by peaks of roughly equal magnitude, was observed in 17.9 % (12/67) of ITD-sensitive neurons. Panel C of Figure 3.1.4 shows the response of a neuron in which positive ITDs evoked roughly the same (near maximal) discharge

rate, whilst negative ITDs evoked few spikes. This **sigmoidal** response type was observed in 16.4 % (11/67) of ITD-sensitive neurons. Panel D of Figure 3.1.4 shows the response of neuron with approximately equal discharge rates at all ITDs, save for a sharp and distinctive trough in the response around 0 ms ITD. Neurons that exhibited troughs in their ITD functions, usually near 0 ms ITD (**trough** response type) accounted for 14.9 % (10/67) of ITD sensitive neurons.

3.1.3 Rate versus Intensity Functions

Changes in the responsiveness of a neuron with the intensity of a binaural click could be categorised into 4 distinct response types. Figure 3.1.5 shows the response of four neurons presented with a binaural click delivered at best ITD over the intensity range 30-85 dB SPL. The majority of neurons (51/83) exhibited either a monotonic increase in their response with increasing intensity that saturated at high intensities (26/83 neurons; **Saturating** response type; Figure 3.1.5, panel A) or a non-saturating monotonic increase in their response with increasing stimulus intensity (25/83 neurons; **Monotonic** response type; Figure 3.1.5, panel B). A further 19/83 neurons displayed a monotonic increase in their response at low intensities followed by saturation of their response at relatively high intensities with a subsequent decrease in responsiveness with further increases in intensity (19/83 neurons; **Non-monotonic** response type; Figure 3.1.5, panel C). The final group of neurons exhibited a monotonic increase at low intensities followed by saturation at medium intensities with a further monotonic increase at high intensities (13/83 neurons; **Saturating/monotonic** response type; Figure 3.1.5, panel D). Figure 3.1.6 shows the distributions of the BFs of neurons classified as having one of the four response types. The histograms of BF distribution in each classification indicate there was no clear relationship between a neurons BF and its rate-vs.-intensity function.

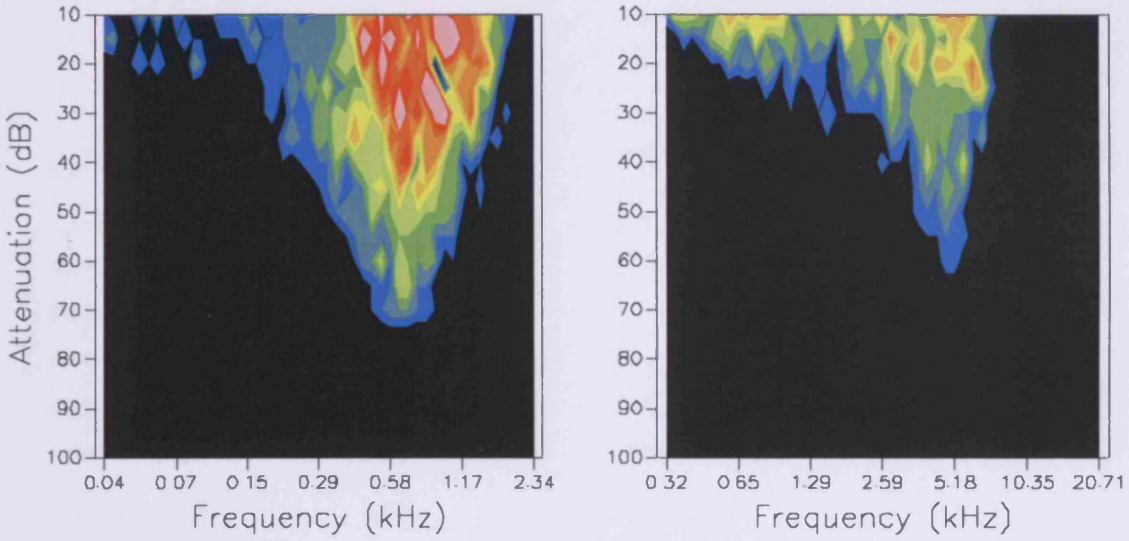


Figure 3.1.1 Responses of two neurons to pure tones of varying frequency (abscissa) and intensity (ordinate). Warm colours indicate higher firing rates. BF was defined as the frequency that elicited a response at the lowest sound intensity.

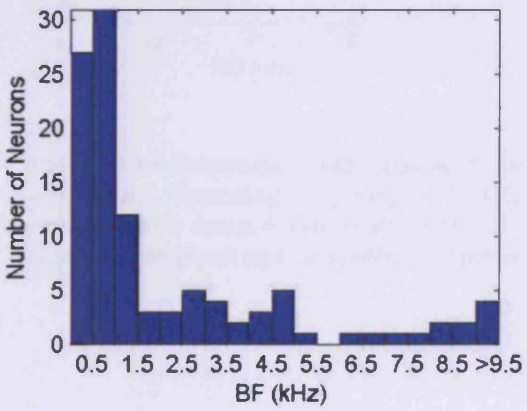


Figure 3.1.2 Distribution of best frequencies (BFs) for sampled neurons (N = 108). The majority of neurons studied had best frequencies below 2 kHz.

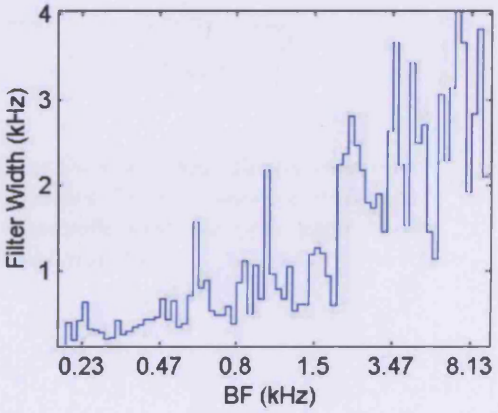


Figure 3.1.3 Frequency-response area widths measured with pure tones at 10 dB re. threshold (N = 108). The 10-dB bandwidth increased with best frequency.

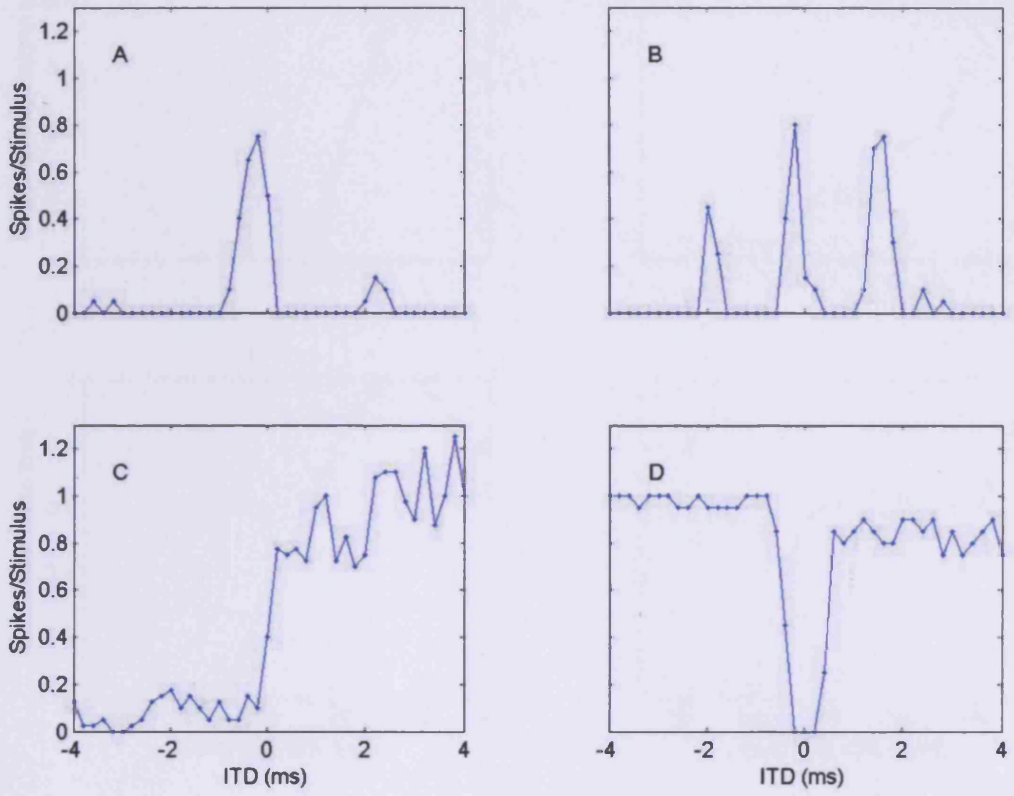


Figure 3.1.4 Responses of four neurons to binaural clicks presented with ITDs of -4ms-4ms (40 repeats) illustrating the variety of ITD functions recorded. Panel A shows a **peak** type response with damped side peaks, panel B shows a **periodic** response type, panel C, a **sigmoidal** response type and panel D a typical **trough** type response.

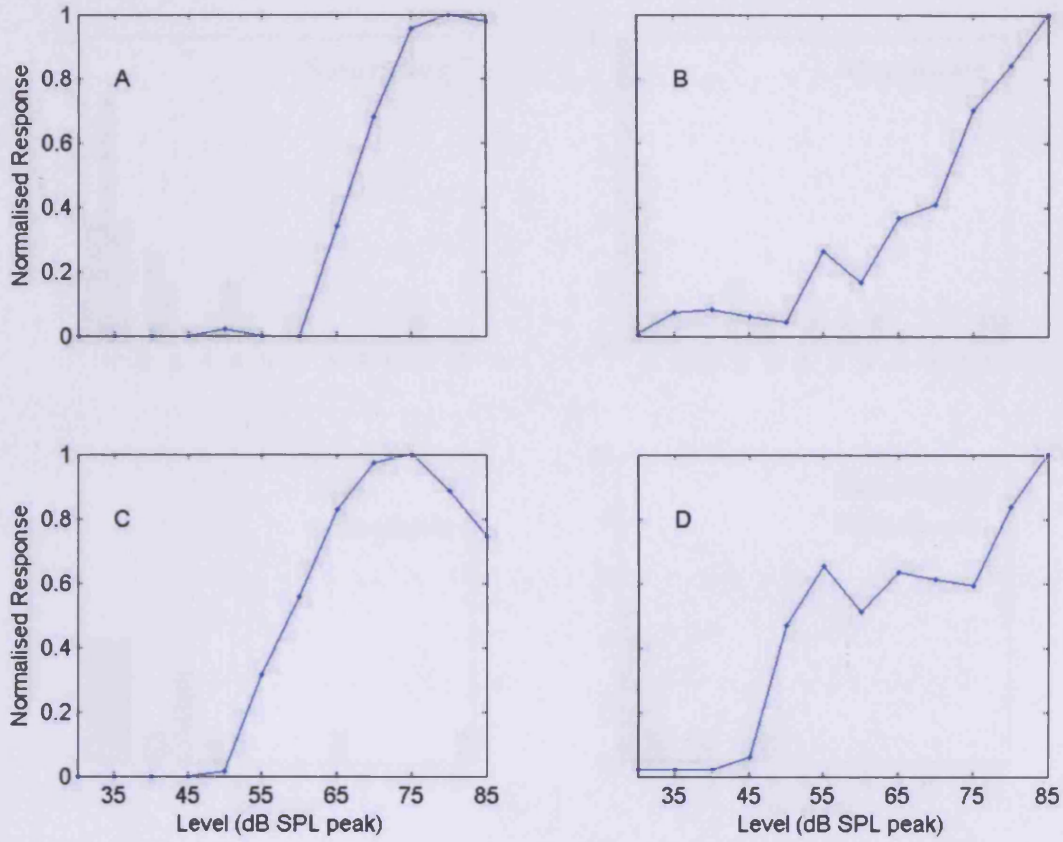


Figure 3.1.5 Responses of four neurons to binaural clicks presented at best ITD at intensities ranging from 35-85 dB SPL peak (40 repeats) illustrating the variety of rate-intensity functions recorded. Panel A shows a **saturating** response type, panel B shows a **monotonic** response type, panel C, a **non-monotonic** response type and panel D a **saturating/monotonic** response type.

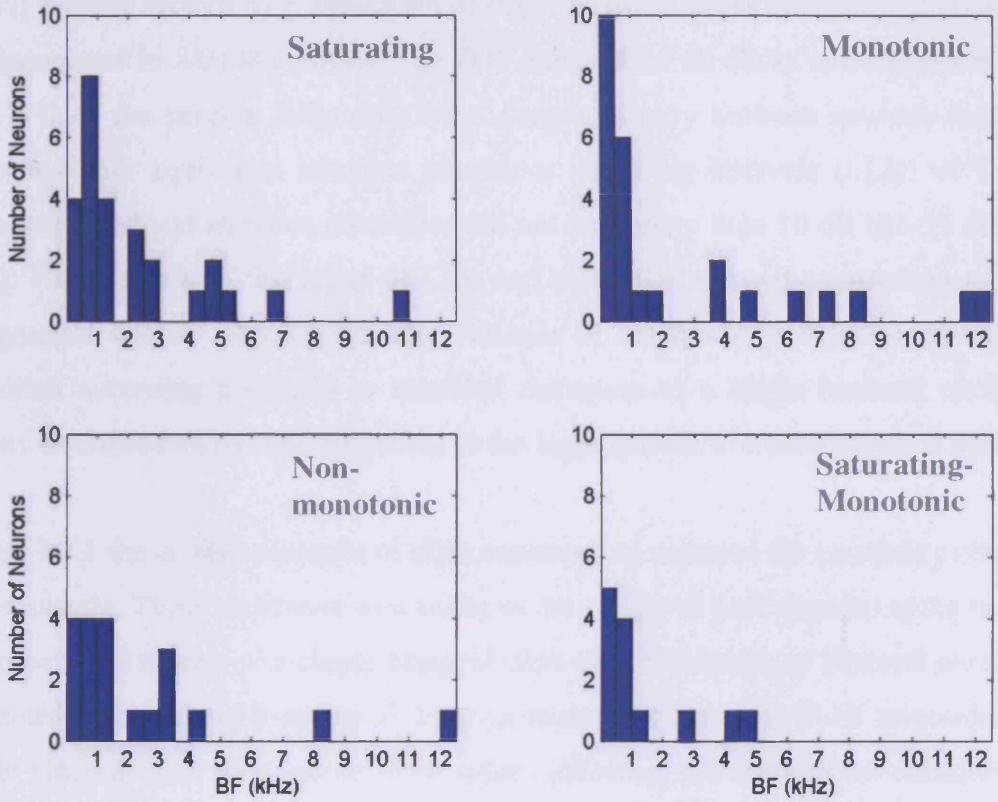


Figure 3.1.6 Histograms showing the distributions of BFs of neurons classified as having **saturating**, **monotonic**, **non-monotonic** and **saturating-monotonic** rate-vs.-intensity functions. The distributions of BF did not show a clear bias for any of the response types.

3.2 Characterisation of Neural Responses to Binaural Clicks

Neural activity evoked by presentation of single binaural clicks and binaural click pairs was compared in 84/108 neurons. The ITD imposed on all clicks corresponded to the best ITD of the neuron. Responses were compared only between neurons that were presented with equivalent stimulus conditions. Lead-lag intervals (LLIs) of 2-10ms were compared and stimulus intensities did not vary more than 10 dB (85-95 dB peak SPL). The selection of this range of LLIs and intensities allowed comparison amongst the greatest number of neurons. The varieties of responses to binaural clicks were classified according primarily to neuronal responses to a single binaural click, and further distinguished by their responses to the lagging click of a binaural click pair.

Figure 3.2.1 shows the responses of eight neurons that captured the variability observed in the sample. These are shown as a series of dot rasters of post stimulus spike times in response to 20 repeats of a single binaural click and in response to binaural click pairs presented with LLIs of 2-10 ms in 2 ms increments. Neurons A-D all responded to a single binaural click with one or more spikes following the onset of the stimulus that, when represented as a dot raster, formed a single continuous period of spike activity that ranged from compact (Neuron A) to dispersed (Neuron D) in appearance. Neuron A responded to a single presentation of a single binaural click with one or two spikes for which the response latency for subsequent presentations of the stimulus was closely synchronised forming a compact looking raster plot. Neurons B to D responded with an increasing number of spikes to a single presentation of the stimulus occurring over longer durations and with progressively poorer onset synchrony between presentations of the stimulus, forming more dispersed looking raster plots. This range of responses was categorized as having a **single** response type because of the single period of spike activity in response to a single binaural click. Two thirds of the neurons assessed (65.5%; 55/84) responded to a single binaural click with a single response type.

For neurons that exhibited the **single** response type, a response to the lagging click was absent in some neurons and present in other neurons for LLIs of less than 10 ms. The response durations of Neuron A, and Neuron C (Figure 3.2.1) both increased at an LLI of 4 ms. In the case of Neuron A the spikes accounting for the increase in the duration of the response were distinct from the response to the leading click and coincided with

the LLI of the stimulus and thus represent the lagging click. The increase in the duration of the response for Neuron C, however, at a LLI of 4 ms resembled an increase in the number of spikes in response to a single binaural click rather than a robust and isolated representation of the lagging click. These spikes eventually became a distinct cluster in the raster plot, presumably indicating the isolated response to the lagging binaural click. At the LLIs for which the lagging click was represented as a distinct period of spike activity (> 4ms), the response to the leading click returned to the same duration as the response to the single binaural click. In contrast to neurons A and C, neurons B and D did not show an increase in response duration when the LLI was less than 10 ms and there was no obvious representation of the lagging click in the raster plots.

The LLI at which spikes began to occur later in the response to binaural click pairs than in the response to a single binaural click appears to indicate the point at which independent representation of the lagging click began for neurons A and C. Neural representation of the lagging click was therefore defined as the LLI at which an increase in the duration of the response to a binaural click pair above that to a single binaural click occurred. Of the neurons that responded with a single response type 60% (33/55) showed a response to the lagging click of a binaural click pair while 40% (22/55) did not. The distribution of BFs for neurons that had a single response type (Figure 3.2.2) reflected that of the entire sample (Figure 3.1.2). Responsiveness to the lagging click for these neurons was not predicted by the neuron's BF, indicating suppression of response to the lagging click operated for neurons of all BFs.

The second response type described was one in which multiple periods of spike activity were apparent in the dot rasters of post stimulus spike times in response to a single binaural click (Figure, 3.2.1, neurons E and F). This **multiple** response type was observed in a quarter (23/84, 27.4%) of neurons. The possibilities that the **multiple** response type was a result of the neuron responding to each monaural click separately when the ITD of the stimulus was long, or constituted an offset response resulting from a high stimulus intensity, were considered. In addressing the first possibility Figure 3.2.3 shows a series of dot rasters of post stimulus spike times in response to a binaural click with ITDs of -4 ms to 0 ms imposed on it. The time at which the second response occurred was not related to the ITD of the stimulus. Figure 3.2.4 shows a raster plot of

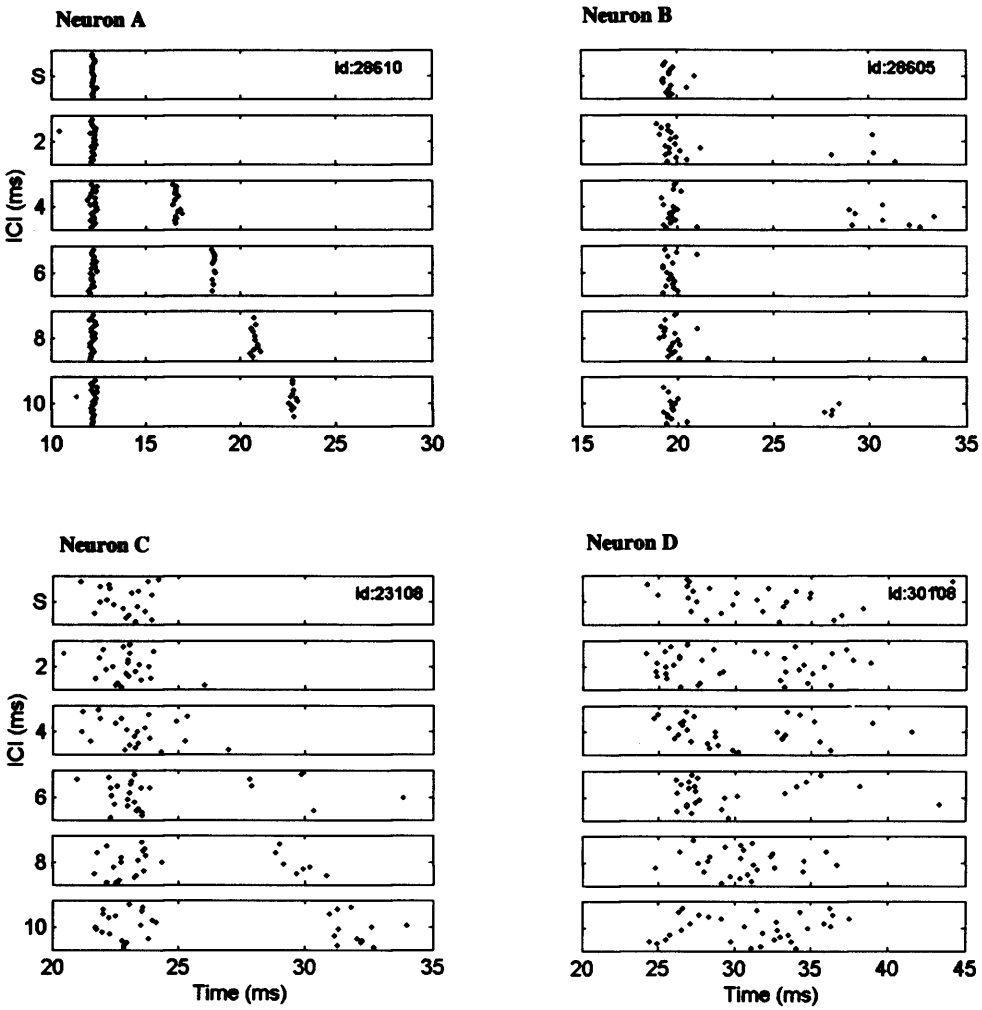
post stimulus spike times for a binaural click of decreasing intensity for the same neuron. The second response was present at both high and low stimulus intensities although it appears to be less prominent at lower intensities indicating it may be related to the stimulus intensity but is not an offset response evoked at high stimulus intensities.

When presented with binaural click pairs the vast majority (21/23, 91.3%) of neurons with a **multiple** response type showed a raster plot with a second tightly-distributed cluster in their dot raster corresponding to the LLI of the stimulus (typified by Neuron E, Figure 3.2.1). The **multiple** response profile was not dependent on neuronal BF (Figure 3.2.2).

The third and fourth response types are described by the response of neurons G and H in Figure 3.2.1. Neuron G was highly spontaneous, and showed a reduced firing rate when click stimuli were presented (**suppressed** response type). The two neurons that exhibited this response type had BFs of 7.7 and 8.6 kHz. Neuron H displayed the fourth response type encountered which was a weak response to the first click and a more robust response to the second click of the click pair (**lag** response type). This response was seen in just 4/84 of the neurons sampled, with these neurons showing BFs across the range recorded.

The duration of the response to a single click in neurons of the auditory nerve is longer for lower BF neurons than higher BF neurons. The reason for this is linked to the duration of the cochlea's response when stimulated with a click. The duration of responses to single binaural clicks in the IC has not previously been shown to correlate with the neurons BF. The duration of the response to a single binaural click for the neurons with a single response type was quantified using the inter quartile range of spikes occurring in the first 50 ms of the post stimulus response. Figure 3.2.5 shows the inter quartile range of the post stimulus spike times in response to a single binaural click for each neuron plotted as a function of the BF of the neuron. The red line indicates the best fit of the data points using coefficients generated by a third order polynomial fitting procedure. The resulting curve indicates that the duration of the response increases as the BF of the neuron decreases. This effect was most pronounced for neurons with BFs below 1 kHz, above which response duration was relatively

homogenous. The slight up-turn in the fitted curve observed toward the extreme high frequencies disappeared when the neurons with the five highest BFs were excluded from the fit suggesting it could be attributed to the small number of neurons in the analysis with high BFs. The distributions of the inter-quartile ranges among the sample are presented in Figure 3.2.6.


Neuron A
Id:28610
S
2
4
6
8
10
ICI (ms)
10
15
20
25
30
Neuron B
Id:28605
15
20
25
30
35
Neuron C
Id:23108
S
2
4
6
8
10
ICI (ms)
20
25
30
35
Time (ms)
Neuron D
Id:30108
20
25
30
35
40
45
Time (ms)

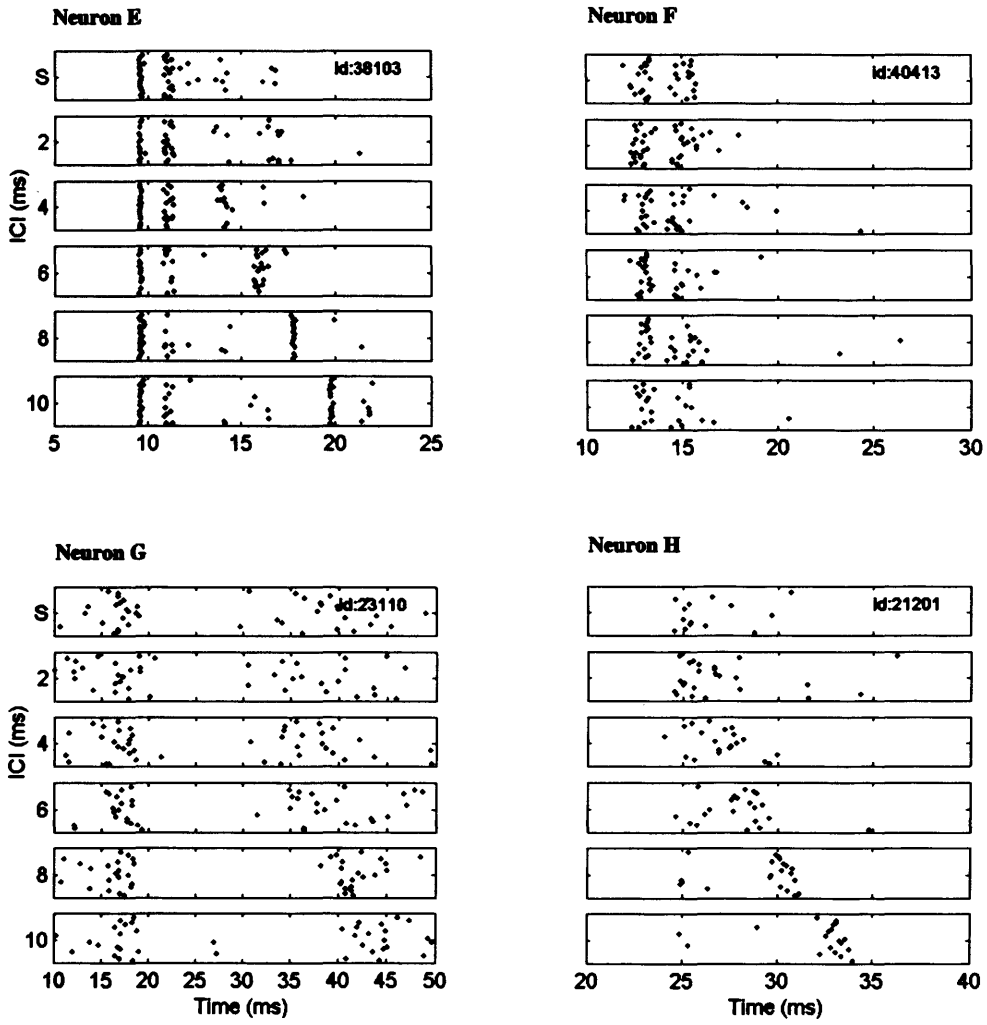


Figure 3.2.1 Dot rasters of spike times showing the responses of 8 different neurons to 20 repeats of a single binaural click (S) and binaural click pairs of 2, 4, 6, 8, and 10 ms LLI presented at best ITD. Neurons A-D shows the variations in the single response type, Neurons E and F the multiple response type, Neuron G the suppressed type and Neuron H the lag response type.

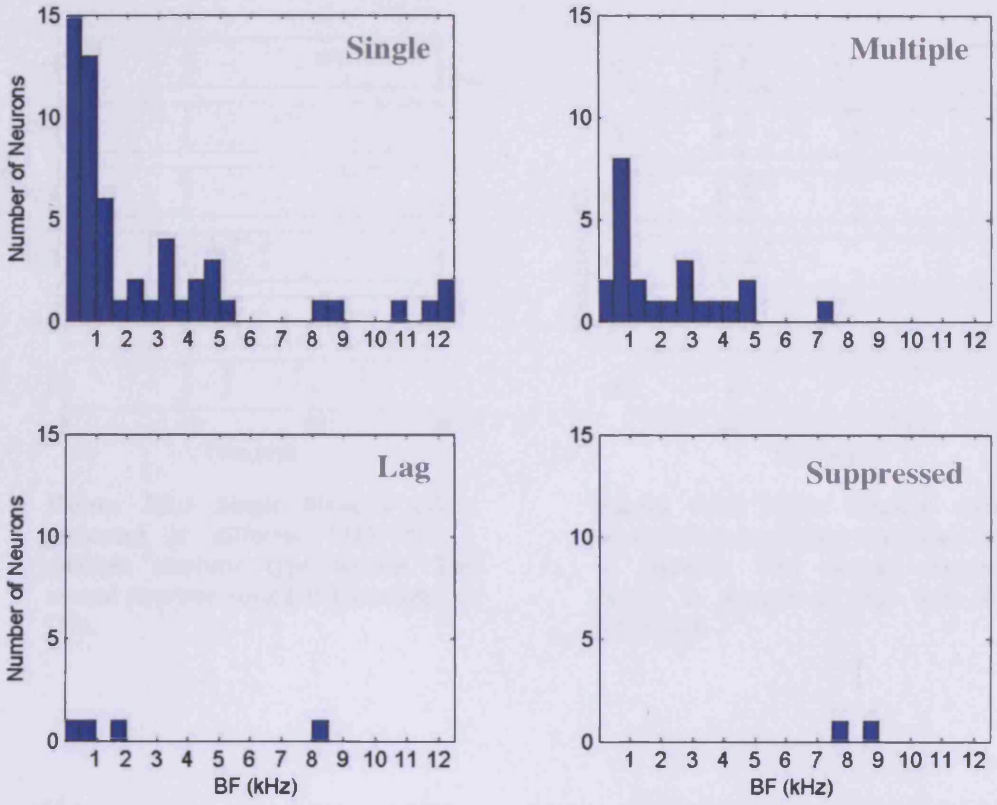


Figure 3.2.2 Histograms showing the distributions of BFs of neurons classified as having single, multiple, lagging or suppressed response types when presented with binaural clicks and binaural click pairs. The distributions of BF did not show a clear bias for any of the response types.

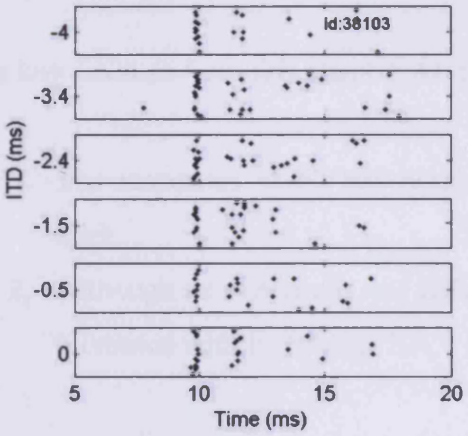


Figure 3.2.3 Single binaural clicks presented at different ITDs for a multiple response type neuron. The second response period is not related to ITD.

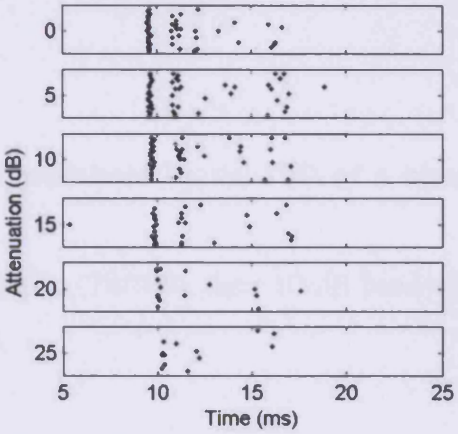


Figure 3.2.4 Single binaural clicks presented at decreasing intensities (top to bottom). The second response period is present at high and low intensities.

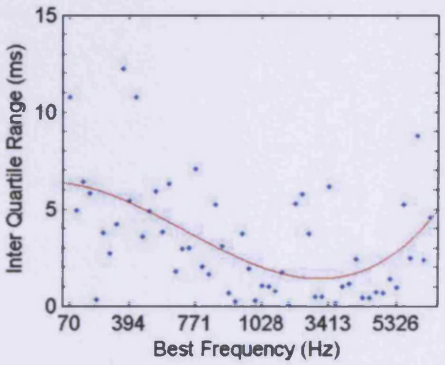


Figure 3.2.5 The inter-quartile range (IQR) of post stimulus spike times in response to a single binaural click plotted as a function of BF. Each point is the response of a different neuron. The line of best fit shows a decrease in IQR with increasing BF (n = 55).

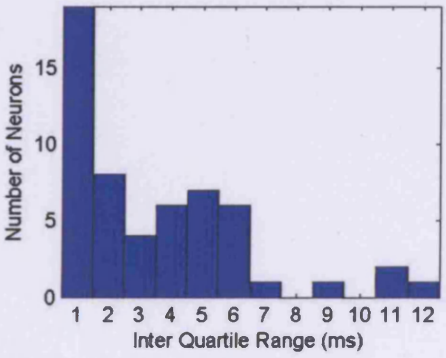


Figure 3.2.6 Distributions of the inter quartile ranges (IQRs) of neurons (n=55).

3.3 Results Part I: Key Findings

The key findings from this chapter were,

1. The responses of 67/108 neurons were modulated by the ITD of a binaural click.
2. Although most neurons had BFs below 2 kHz (78/108), their 10-dB bandwidths increased with increasing BF.

4.0 Results Part II:

Neural Responses to Leading and Lagging Clicks

Data presented in the this chapter describe neural responses to binaural click pairs presented with the same ITD and at equal intensity. For neurons sensitive to the ITD of a binaural click, the leading and lagging clicks were presented at the best ITD of the neuron while for neurons insensitive to the ITD of a binaural click leading and lagging clicks were presented with ITDs of 0 ms (diotic presentation). Responses recorded from 80 neurons located in the IC of the guinea pig with BFs ranging from low to high were analysed. The neurons comprising the sample came from one of three groups in which the stimulus intensity, lead-lag intervals (LLIs) and the range of BFs of neurons varied. For each neuron the response to the leading click of a binaural click pair was compared with the response to the same click presented alone as a function of the LLI. In addition, the response to the lagging click was compared with the response to the same click presented alone as well as the response to the leading click of the binaural click pair as a function of the LLI. Responses to the leading and lagging click were calculated by counting the number of spikes occurring in corresponding response windows (see Methods, section 2.5.6) and expressed as a percentage of the response to the comparator click, as a function of the LLI.

4.1 Modulation of Neural Responses to Leading and Lagging Clicks as a Function of the Lead-Lag Interval

Neural responses to the leading click of a binaural click pair were enhanced or suppressed by the lagging click. For some neurons the effect depended on the LLI of the stimulus. For all neurons the leading click suppressed responses to the lagging click. The degree of suppression depended in the LLI of the stimulus. Figure 4.1.1 shows the response of a low-BF (1 kHz) neuron for which the lagging click suppressed the response to the leading click and the leading click suppressed the response to the lagging click at all LLIs examined. The PSTHs presented in the top 11 panels of the figure with the leading and lagging response windows plotted in red and black respectively, show the neurons response to a single binaural click and binaural click pairs separated by LLIs of 2-20 ms presented at best ITD and at an intensity of 50 dB peak SPL (40 repeats). The lead response plot (bottom left panel) shows the response of the neuron to the leading click calculated from the leading click response window (red lines on the PSTHs). The blue line with open circles represents the number of spikes occurring during the entire leading click window for LLIs of 2-20 ms expressed as a percentage of the number of spikes occurring during the equivalent window applied to the response to a single binaural click. The values indicated by the black line with dots were calculated in the same way as those indicated by the blue line for LLIs that did not produce overlapping response windows. For LLIs that produced overlapping response windows i.e. 2 ms, the end of the leading click response window coincided with time of arrival of the lagging click, indicated by the first black line on the PSTH. The lag response plot (panel on the bottom right of figure 4.1.1) shows the response of the neuron to the lagging click as a percentage of the response to the leading click i.e. the recovery function of the response to the lagging click. The black line with dot markers shows the recovery function for LLIs where the response windows didn't overlap, where the windows overlapped the values were set to 0. The blue line shows the recovery function including LLIs for which the response windows overlapped and the response to the leading click was estimated (see Methods, section 2.5.6).

The PSTHs show the neuron responded to the leading click at all LLIs and that the response varied as a function of LLI as indicated by changes to the distribution and

number of spikes occurring in each leading click response window. At 2-ms LLI, the duration of the response was the same as that to a single binaural click however, the change in spike distribution was accompanied by a shift in the neurons response latency. The shift in response latency meant part of the response occurred after the arrival of the lagging click indicated by the overlap in the response windows. As such, it is not clear whether this was a response to the leading click or a response to both clicks. The black line on the lead response plot (bottom left panel, Figure 4.1.1) shows the neurons response to the leading click was suppressed by the lagging click for LLIs of 2-20 ms and as the LLI increased the suppressive effect of the lagging click reduced. For an LLI of 2 ms the response to the leading click was 28% of the response to a single binaural click (equivalent window) and by 20-ms LLI, the response had recovered to only 67% of the response to a single binaural click. For this example, the neurons response during the entire leading click window i.e. over the same duration as the response to a single binaural click (blue line, open circles) was also suppressed for all LLIs despite the arrival of the lagging click during the response window for an LLI of 2-ms. The neuron began responding to the lagging click at LLIs of 4-20 ms evident in the lagging click response window plotted on the PSTHs (Black dotted lines, Figure 4.1.1). For this neuron both recovery functions shown in the lag response plot (bottom right panel, Figure 4.1.1) indicated the same result, the neurons response to the lagging click recovered monotonically despite the variability in the neurons response to the leading click and reached 50% recovery at an LLI of 13.13 ms. It should be pointed out that the recovery function calculated according to Tollin et al. (2004) does not describe the recovery of the neurons response to the lagging click for overlapping response windows when the response to the leading click is variable. For these LLIs the recovery function indicates the increase in spike activity relative to the response to the comparator click but the spikes can not be attributed to the leading or lagging click with certainty.

Figure 4.1.2 shows the response of a second low-BF (1.03 kHz) neuron for which the lagging click generally enhanced the neurons response to the leading click depending on the LLI, and like the previous neuron the leading click suppressed the response to the lagging click at all LLIs. Figure 4.1.2 follows the same format as the previous example and shows the response of the neuron to a single binaural click and binaural click pairs separated by LLIs of 2-20 ms presented at best ITD and at an intensity of 55

dB peak SPL (40 repeats). The PSTHs show the neuron responded to the leading click (red lines) at all LLIs however, the response at an LLI of 4 ms totalled 1 spike for 40 presentations of the stimulus compared with 15 spikes in response to a singe binaural click. As for the previous neuron, the PSTHs of the response of this neuron indicate a shift in response latency occurred at an LLI of 2 ms. In contrast to the previous neuron, the lagging click had the effect of suppressing the response to the leading click when the LLI was 2 or 4 ms after which the lagging click *enhanced* the response to the leading click (black line, Lead Response plot, Figure 4.1.2). The enhancive effect of the lagging click resulted in the neuron responding with between 47% (LLI = 16 ms) and 113% (LLI = 14 ms) more spikes than in response to a single binaural click depending on the LLI. A reliable response to the lagging click for this neuron emerged at an LLI of 12 ms and was present for all subsequent LLIs. The recovery functions for the neuron presented in the lag response plot (bottom right panel of figure 4.1.2) both show the response to the lagging click to have recovered to twice the magnitude of the response to the leading click at a LLI of 4 ms. It is apparent from the PSTH for the corresponding LLI that this is a spurious value due to the low number of spikes evoked for this stimulus condition. Ignoring the LLIs of 2-4 ms both recovery functions indicate the response of the neuron to the lagging click recovered to 50% of the response to the leading click at an LLI of 15.36 ms, however for subsequent LLIs the recovery function indicates the response returned slightly below 50% due to the LLI dependent backward enhancement of the response to the leading click.

Figure 4.1.3 shows the response of a third low-BF (780 Hz) neuron for which the lagging click periodically suppressed the response to the leading click and for which the leading click suppressed the response to the lagging click also in a periodic manner. This example shows the responses of the neuron to a single binaural click and binaural click pairs separated by LLIs of 2-20 ms presented at best ITD, and at an intensity of 45 dB peak SPL (40 repeats). The PSTHs indicate the neuron responded to the leading click (red lines) at all LLIs with the spike distribution and magnitude depending on the LLI. For this neuron, there was no characteristic backward masking or enhancement present at most LLIs as was the case for the previous two examples. Rather, for LLIs of 6, 14, and 20 ms the response was equivalent to the response to a single binaural click and for all other LLIs the response was suppressed (black line, Lead Response plot, Figure 4.1.3). The periodicity of the suppression did not appear to be regular as

indicated by the uneven number of points between each peak and trough of the black line on the lead response plot. The response during the entire leading click window (blue line, Lead Response plot, Figure 4.1.3) was 122% of the response to a single binaural click for an LLI of 2 ms and 83% of the response to a single binaural click for an LLI of 4 ms as opposed to 69% and 50% prior to arrival of the lagging click (black line). Both of these values indicate the response during the same period as the response to a single binaural click followed the same pattern as the response attributable to only the leading click. The neuron responded to the lagging click for LLIs of 2 ms onward although a distinct response did not emerge until 8-ms LLI (PSTHs, Figure 4.1.3). The recovery functions presented in the bottom right panel of Figure 4.1.3 also display a periodic pattern influenced by the response to the leading click. The blue line on the lag response plot shows the neuron responded with more than double the number of spikes it did to a single binaural click for an LLI of 2 ms however for an LLI of 20 ms where the response to the leading click was 100% of the response to a single binaural click the recovery of the response to the lagging click had reduced to 53%. The recovery function calculated from LLIs for which the response windows did not overlap (black line, Lag Response window) indicates 50% recovery occurred at an LLI of 5.2 ms. The recovery function calculated according to Tollin et al. 2004 (blue line) indicates 128% recovery for an LLI of 2 ms and 44% for an LLI of 4 ms.

Figure 4.1.4 shows a fourth low-BF (370 Hz) neuron for which the lagging click had little effect on the neurons response to the leading click and which the leading click suppressed the response to the lagging click for all LLIs. The PSTHs indicate the neuron responded robustly to the leading click (red lines) at all LLIs. The lead response plot (bottom left panel, Figure 4.1.4) indicates that with the exception of 4-ms and 18-ms LLI the neurons response to the leading click was within 10% of its response to a single binaural click for the remaining LLIs. For 4 and 18ms LLI the response was 71% and 84% of the response to a single binaural click respectively. Due to the long duration of the neurons response to a single binaural click (11.3 ms), the leading response window prior to arrival of the lagging click (first black line, PSTHs) represents a relatively small proportion of the response to a single binaural click. However the spike count during the entire leading click window (blue line, Lead Response plot, Figure 4.1.4) was also stable varying between 84% (LLI = 18ms) and 108% (LLI = 16ms) with the exception of 4-ms LLI for which the response was 129%

of the response to a single binaural click. The PSTHs (Figure 4.1.4) indicate the neuron responded with a greater number of spikes to a binaural click pair than to a single binaural click for all LLIs with a distinct response to the lagging click emerging at an LLI of 10 ms. For this neuron the lag response plot (bottom right panel) shows that for LLIs of 2-10 ms the neuron responded with greater than 50% of the spikes that it did to a single binaural click (blue line, open circles) although there was no distinct response to the leading and lagging click. For the response windows that didn't overlap (> 12-20 ms) a distinct representation of the leading and lagging clicks is apparent, and the response to the lagging click recovered to 60% at 12 ms LLI. Despite the stability of the response to the leading click the neuron still exhibited a non-monotonic recovery function with the response to the lagging click reducing to 43% of the response to the leading click for an LLI of 14 ms. For all subsequent LLIs the neuron showed a monotonic increase in its response to the lagging click.

4.1.1 Neural Responses to the Leading Click

The 80 neurons were separated according to the effect the lagging click had on their response to the leading click for the majority of LLIs > 4ms, one of suppression (> 30%, Figure 4.1.1), enhancement (> 30%, Figure 4.1.2), suppression and enhancement (> 30%, Figure 4.1.3), or a minimal effect (< 30%; Figure 4.1.4). While enhancive and suppressive effects of the lagging click were easy to distinguish visually, the difference between a response to the leading click that was not affected by the lagging click (minimal category) and one where there was both an enhancive or suppressive effect depending on the LLI of the stimulus was not. For the purpose of categorising responses, neurons were classified as belonging to the minimal category if their response to the leading click did not deviate by greater than 30% of their response to a single binaural click for LLIs greater than 2 ms. Figure 4.1.5 shows the responses of all neurons to the leading click of a binaural click pair as a function of the LLI. Each of the three groups of neurons (A, B, & C) which were presented with single binaural clicks and binaural click pairs with different ranges of LLIs (Group A, LLIs of 2-20 ms, Group B, LLIs of 2-10 ms, Group C, LLIs of 2-5 ms) and intensities (Group A, 40-70 dB peak SPL, Groups B & C, 85-95 dB peak SPL) are presented separately. Of the 80 neurons 55 showed an effect of the lagging click on their response to the leading click. Twenty five of the 80 neurons showed less than 30% change in their response to the

leading click for the majority of LLIs (Figure 4.1.5, panels labelled minimal). The lagging click enhanced the response to the leading click at most LLIs for 27/80 of the neurons (Figure 4.1.5, panels titled enhance). For 21/80 neurons the lagging click suppressed the response to the leading click for the majority of LLIs (Figure, 4.1.5, panels titled suppress). For 18/80 neurons the lagging click either enhanced or suppressed the response to the leading click by more than 30% depending on the LLI for more than a single LLI (Figure 4.1.5, panels titled (enhance/suppress). While the proportions of neurons for which the lagging click had either a suppressive, enhancive, both suppressive or enhancive effect, or a minimal effect varied between the groups the lagging click effected the response to the leading click by more than 30% for over half of the neurons in each group indicating the lagging click effected responses to the leading click over a broad range of intensities (40-95 dB peak SPL).

The mean and standard deviation of the percentage change in the response to the leading click for all LLIs was calculated to compare the effect of the lagging click on the response to the leading click between neurons within each group. For the groups of neurons presented in the Figure 4.1.5 for which the lagging click either generally suppressed or enhanced the response to the leading click, the mean reflects the consistency of this effect as a function of the LLI. The standard deviation captures the LLI dependence of suppression or enhancement as well as LLI specific suppressive or enhancive effects of the lagging click for neurons in which the there was no shift in the mean. Figure 4.1.6 shows mean and standard deviation for each neuron plotted as a function of BF (top, panels) and firing rate (middle panels). As each group (A, B, & C) had a different range of LLIs, each group of neurons was plotted separately. Furthermore, it would be expected that the effect of the lagging click would decrease with increasing LLI of the binaural click pair and, as such, any BF dependent or firing rate dependent effect would be most apparent for the group of neurons presented with the smallest range of LLIs. The distributions of BFs for each group are presented in the bottom panel of each column of three plots. There was no obvious relationship between a neuron's BF or its firing rate with the variability of its response to the leading click for neurons presented with binaural click pairs separated by LLIs of 2-5 ms (group C), 2-10 ms (Group B) or 2-20 ms (Group A). This finding suggests that neurons with the range of BFs examined can be equally variable in their response to the leading click of a binaural click pair and that neurons with both robust and weak responses to clicks are

equally effected. A non-parametric one-way ANOVA (Kruskal-Wallis) revealed no significant difference between the groups (chi-square = 0.96, p = 0.62) suggesting that effect of the lagging click was robust despite the differences in the range of LLIs and intensities between the groups.

To compare across all neurons examined, average changes in the response to the leading click relative to their response to a single binaural click were calculated for LLIs up to 4 ms and the responses of neurons from each group combined. The mean and standard deviation of the percentage change in response to the leading click for LLIs up to 4 ms is plotted for each neuron as a function of BF and spikes per stimulus (single binaural click) in Figure 4.1.7. For the sample of neurons (N = 80) the effect of the lagging click on responses to the leading click was not predicted by either the neurons BF or the number of spikes evoked by presentation of a single binaural click delivered at best ITD.

For LLIs of up to 4 ms which fall within echo threshold for human listeners (~5 ms) the magnitude of the effect of the lagging click on responses to the leading was quantified for all neurons in the sample. Figure 4.1.8 shows the mean percentage change in response to the leading click (top panel) and the standard deviation of the mean percentage change in response to the leading click (bottom panel) for the sample of neurons. For 30/80 (37.5%) neurons the lagging click produced less than a 10% increase or decrease in the response to the leading click when averaged for LLIs up to 4 ms. For 20/80 (25%) neurons there was between 10-19% suppression or enhancement of the response to the leading click, for 13/80 (16.25%) neurons there was between 20-30% enhancement or suppression of the response to the leading click, while for 17/80 (21.25%) there was greater than 30% suppression or enhancement relative to the response to a single binaural click when averaged for LLIs up to 4 ms. The dependence of the effect of the lagging click on the leading click as a function of the LLI for 34/80 (45.5%) neurons was less than 10% of the response to a single binaural click for LLIs up to 4 ms, between 10-20% for 22/80 (27.5%) neurons, between 20-30% for 13/80 (16.25%) neurons, and greater than 30% for 11/80 (13.75%) neurons.

4.1.2 Neural Responses to the Lagging Click

Recovery functions of responses to the lagging click of a binaural click pair are shown for each group of neurons (A, B & C) in Figure 4.1.9. The panels on the left side show the recovery functions calculated for all LLIs i.e. including overlapping response windows (see Methods section 2.5.6), while those on the right show the recovery functions for LLIs that did not produce an overlap in the response windows. For the three groups of neurons the recovery functions on the left side show a range of effects some of which indicate greater recovery at short LLIs followed by a decline in recovery suggesting the leading click was less suppressive at short LLIs and gradually became more suppressive at longer LLIs. Other neurons show a gradual and often non-monotonic increase in their recovery functions with increasing LLI suggesting the leading click to be generally more suppressive at shorter LLIs. The recovery functions on the right side of Figure 4.1.9 for which only LLIs where the response windows did not overlap were included, all neurons show a non-monotonic increase in their recovery functions with increasing LLI, suggesting that the leading click was more suppressive for shorter LLIs.

Figure 4.1.10 shows the mean recovery function for each group of neurons plotted in the left column of panels, and the recovery to the lagging click at maximum LLI as a function of BF for each group of neurons in the right column of panels. The black lines plotted in the recovery function panels were calculated using overlapping windows, the blue lines without. For all groups of neurons the recovery functions calculated using LLIs that resulted in overlapping windows shows the effect of the leading click to be more suppressive at shorter LLIs than at longer LLIs, with greater than 50% recovery occurring at LLIs of 2 ms. Therefore, the recovery function calculated including overlapping response windows appear unsuitable for this sample of neurons. The recovery function calculated without using LLIs that resulted in overlapping windows shows 50% recovery occurred at an LLI of 16 ms for group A (n = 28), did not occur below the maximum LLI of 10 ms (32.4% recovery) for group B (n = 17), while for group C (n = 7) 50% recovery occurred at an LLI of 4.4 ms. The panels on the right side of Figure 4.1.10 indicate that suppression of response to the lagging click of a binaural click pair for the maximum LLI presented was not predicted by the BF of the neuron for any of the three groups of neurons.

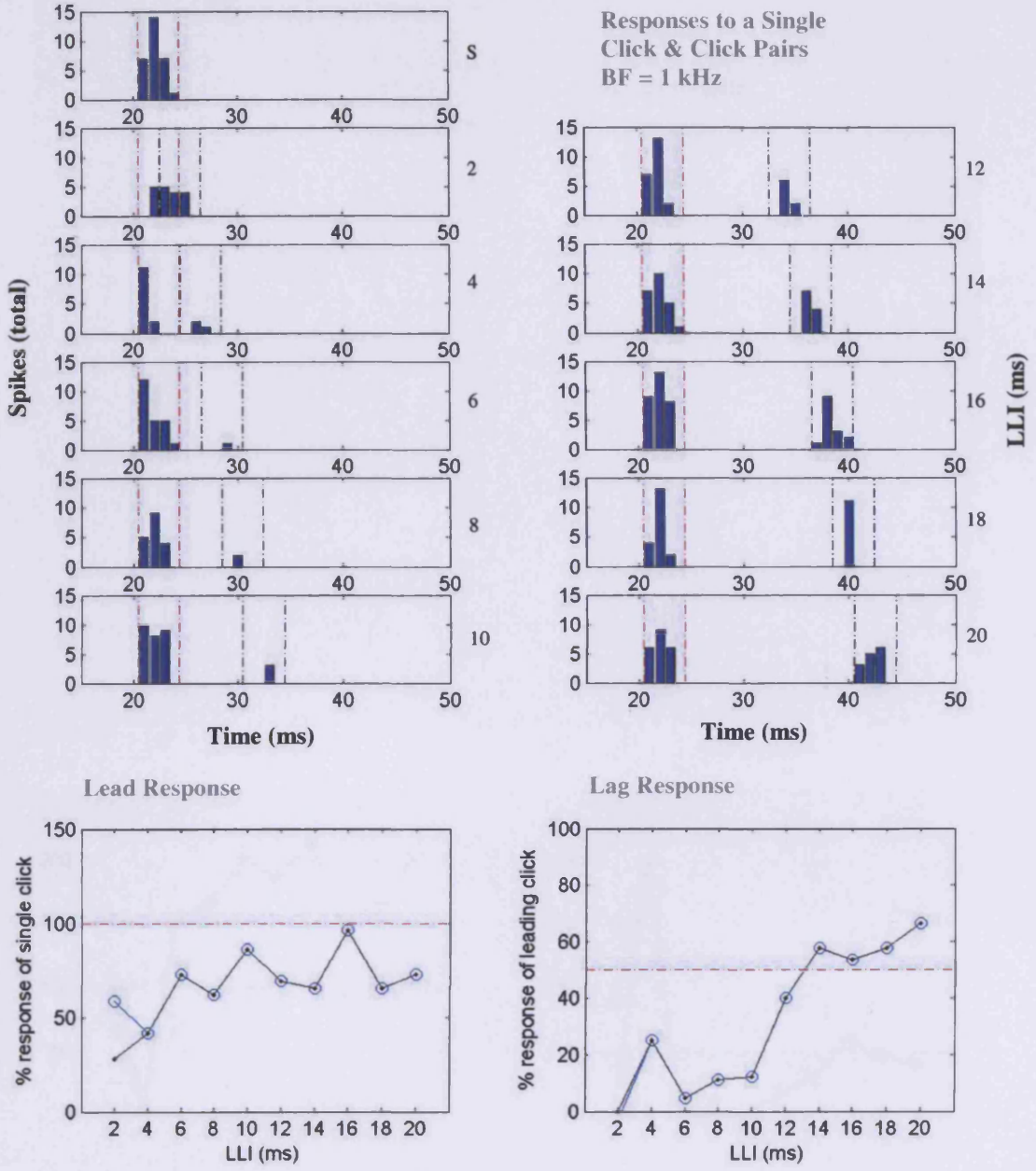


Figure 4.1.1 Responses of a low-BF neuron to a single binaural click (S) and binaural click pairs separated by LLIs of 2-20ms, presented at best ITD and at an intensity of 50 dB peak SPL (40 rpts). The top 11 panels show PSTHs of the responses (1-ms bins). The red lines indicate the leading click window and the black lines the lagging click window. The bottom left panel shows the response occurring in the leading click window (blue line, open circles), and in the leading click window prior to arrival of the lagging click (black line), for each LLI as a percentage of the response to a single binaural click (equivalent window). The bottom right panel shows the response to the lagging click as a percentage of the response to the leading click, with the approximation calculation for overlapping windows (blue line, open circles), and the response to the lagging click only for LLIs for which there was no overlap (black line).

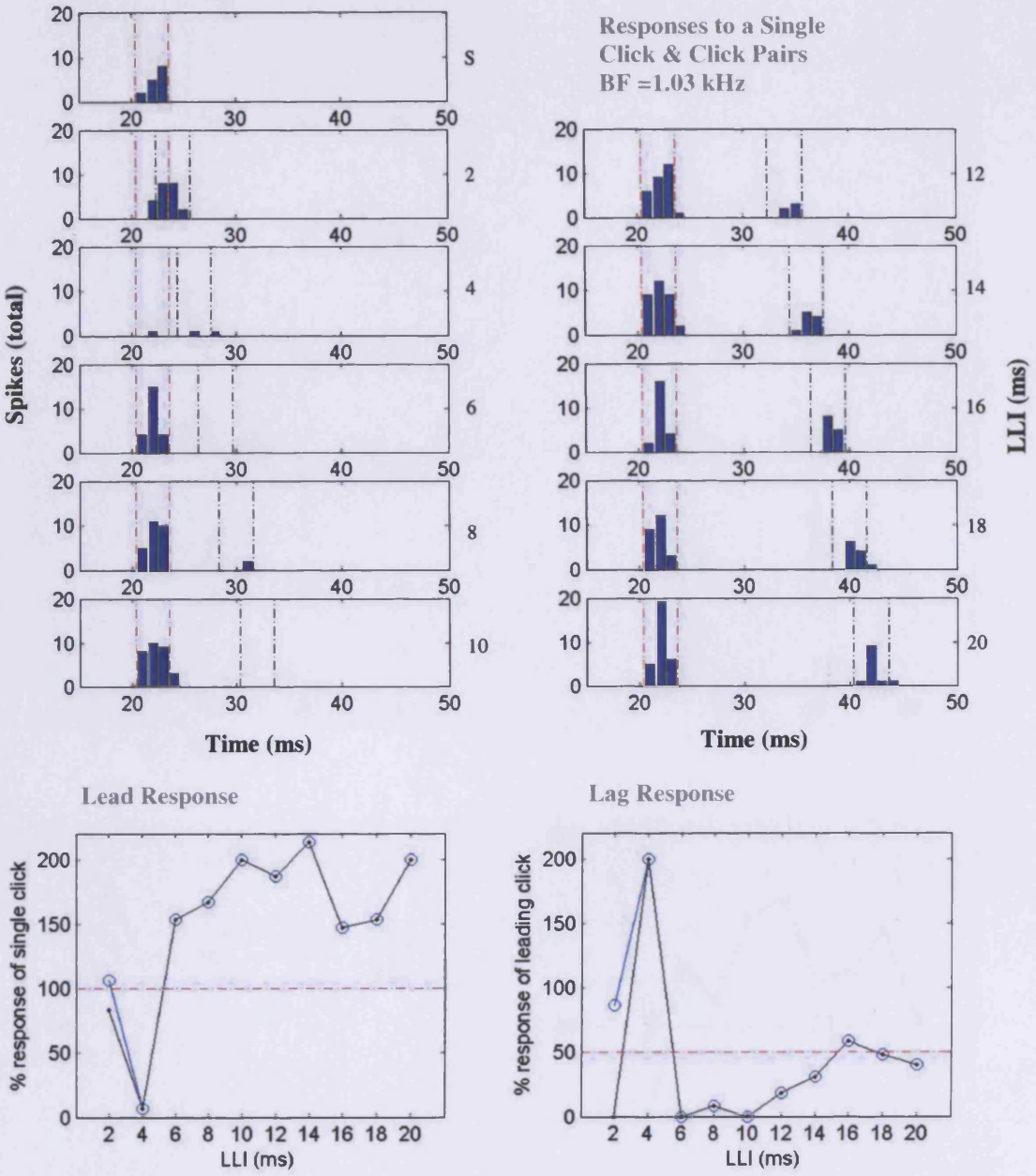


Figure 4.1.2 Responses of a low-BF neuron to a single binaural click (S) and binaural click pairs separated by LLIs of 2-20ms, presented at best ITD and at an intensity of 55 dB peak SPL (40 rpts). The top 11 panels show PSTHs of the responses (1-ms bins). The red lines indicate the leading click window and the black lines the lagging click window. The bottom left panel shows the response occurring in the leading click window (blue line, open circles), and in the leading click window prior to arrival of the lagging click (black line), for each LLI as a percentage of the response to a single binaural click (equivalent window). The bottom right panel shows the response to the lagging click as a percentage of the response to the leading click, with the approximation calculation for overlapping windows (blue line, open circles), and the response to the lagging click only for LLIs for which there was no overlap (black line).

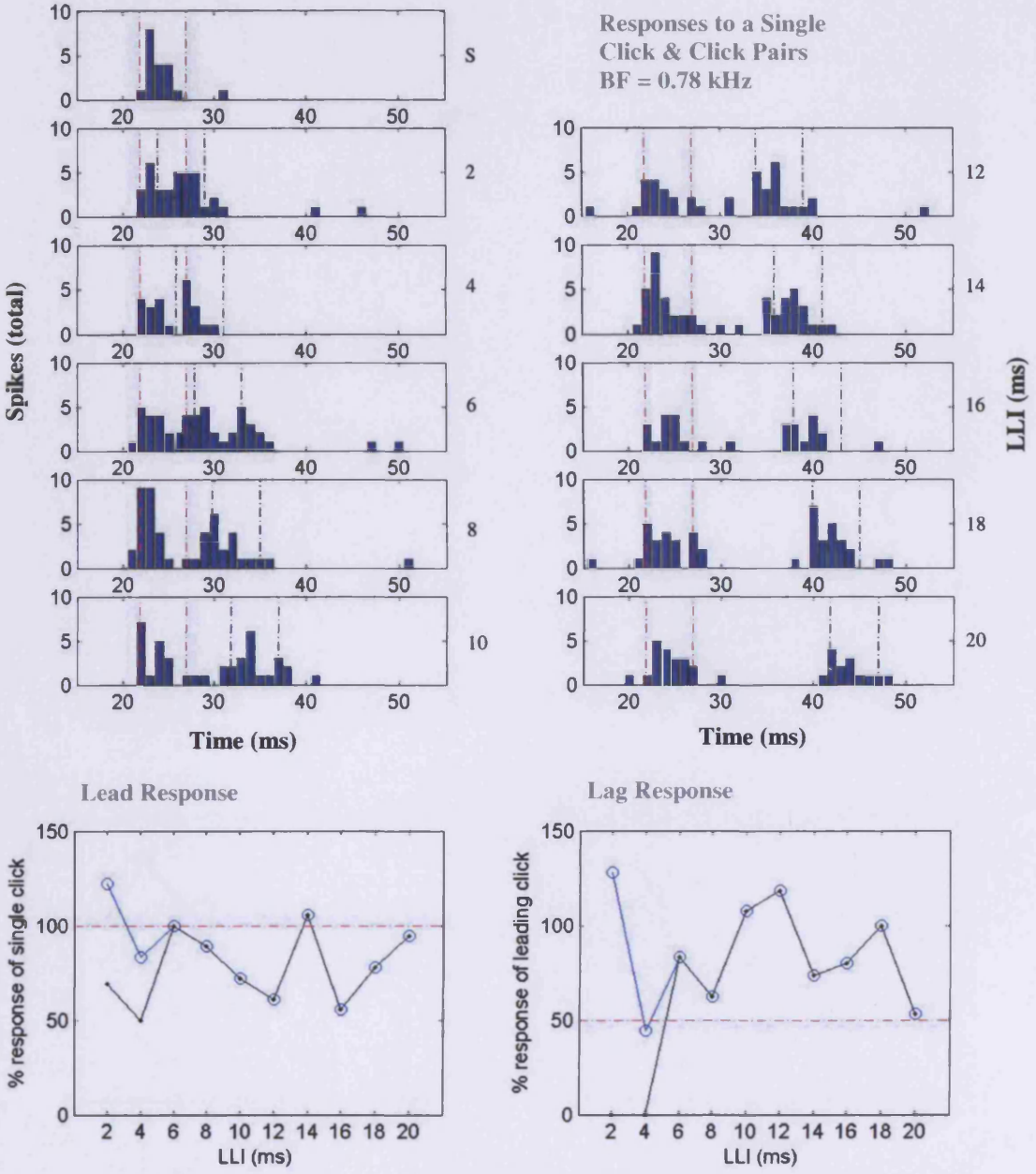


Figure 4.1.3 Responses of a low-BF neuron to a single binaural click (S) and binaural click pairs separated by LLIs of 2-20ms, presented at best ITD and at an intensity of 45 dB peak SPL (40 rpts). The top 11 panels show PSTHs of the responses (1-ms bins). The red lines indicate the leading click window and the black lines the lagging click window. The bottom left panel shows the response occurring in the leading click window (blue line, open circles), and in the leading click window prior to arrival of the lagging click (black line), for each LLI as a percentage of the response to a single binaural click (equivalent window). The bottom right panel shows the response to the lagging click as a percentage of the response to the leading click, with the approximation calculation for overlapping windows (blue line, open circles), and the response to the lagging click only for LLIs for which there was no overlap (black line).

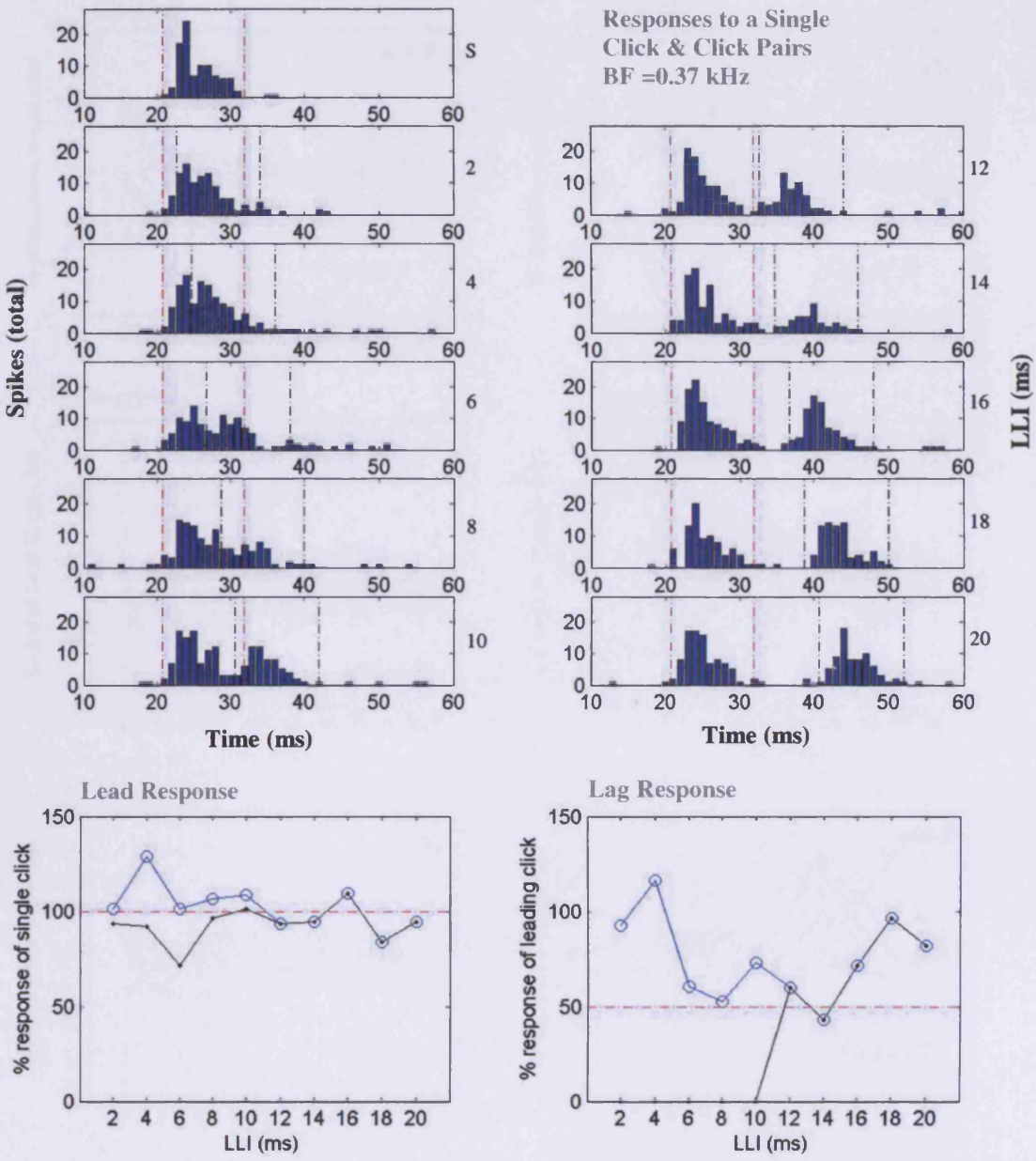
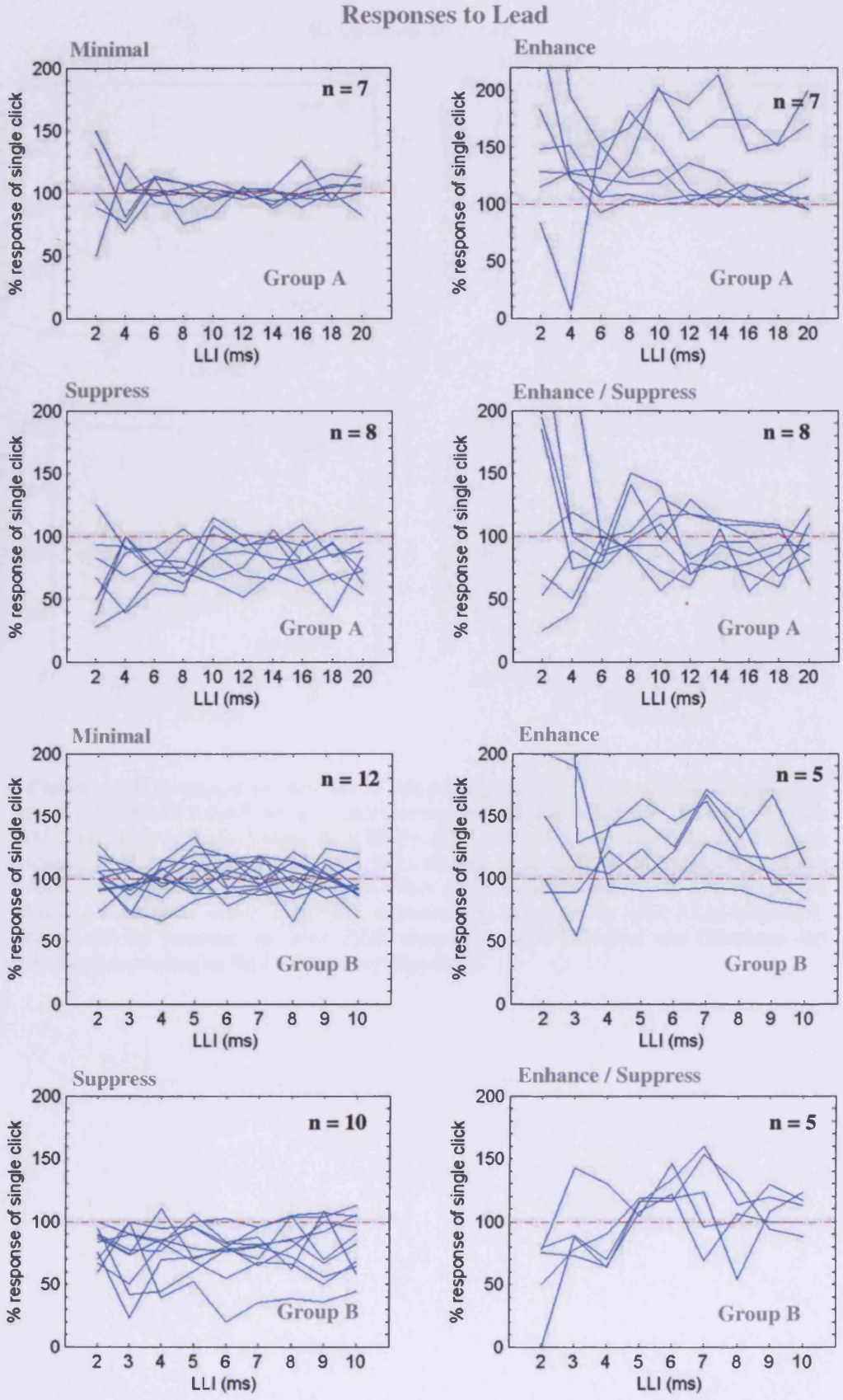


Figure 4.1.4 Responses of a low-BF neuron to a single binaural click (S) and binaural click pairs separated by LLIs of 2-20ms, presented at best ITD and at an intensity of 55 dB peak SPL (40 rpts). The top 11 panels show PSTHs of the responses (1-ms bins). The red lines indicate the leading click window and the black lines the lagging click window. The bottom left panel shows the response occurring in the leading click window (blue line, open circles), and in the leading click window prior to arrival of the lagging click (black line), for each LLI as a percentage of the response to a single binaural click (equivalent window). The bottom right panel shows the response to the lagging click as a percentage of the response to the leading click, with the approximation calculation for overlapping windows (blue line, open circles), and the response to the lagging click only for LLIs for which there was no overlap (black line).


Responses to Lead
Minimal
n = 7
Group A
Enhance
n = 7
Group A
Suppress
n = 8
Group A
Enhance / Suppress
n = 8
Group A
Minimal
n = 12
Group B
Enhance
n = 5
Group B
Suppress
n = 10
Group B
Enhance / Suppress
n = 5
Group B
% response of single click
LLI (ms)

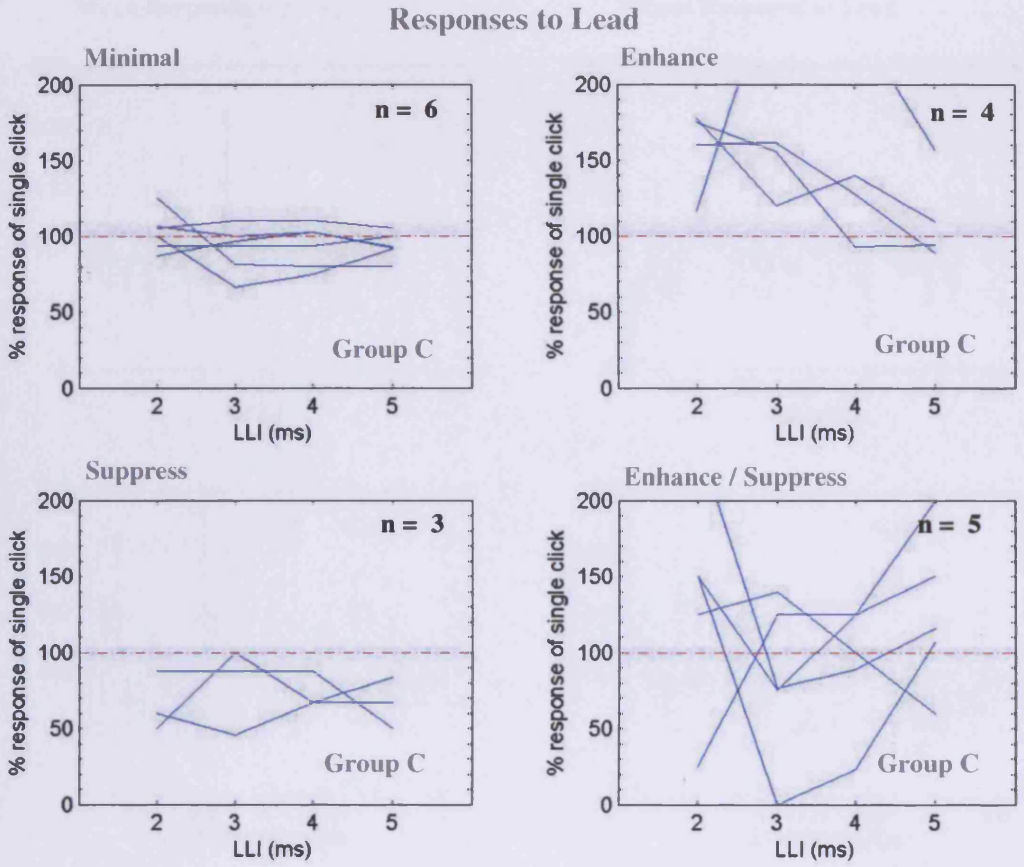
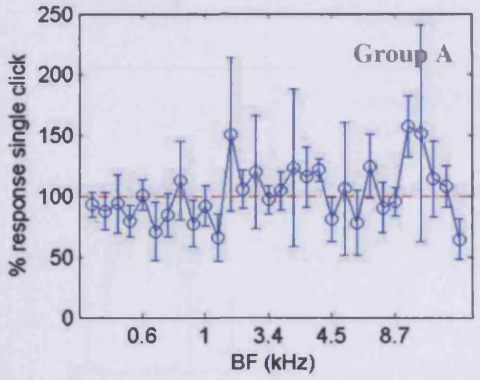
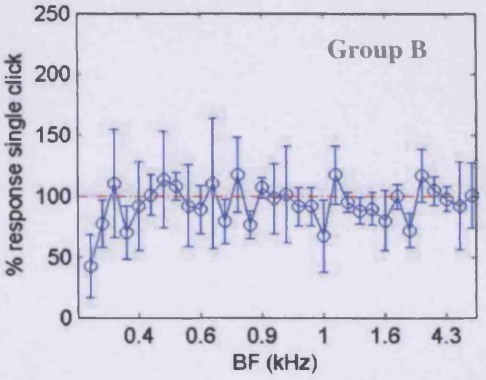


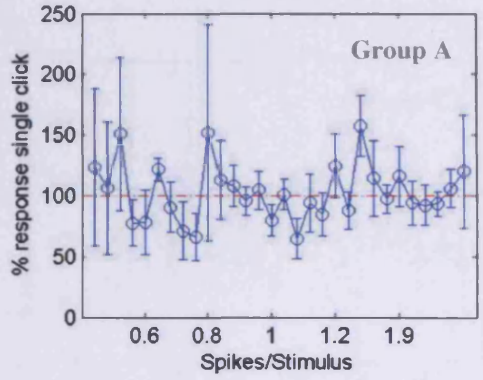
Figure 4.1.5 Responses of neurons to the leading click of a binaural click pair as a function of the LLI. Each group of neurons was presented with binaural click pairs with a different range of LLIs (Group A, 2-20 ms, Groups B, 2-10 ms Group C, 2-5 ms) and intensities (Group A, 40-70 dB peak SPL, Groups B & C, 85-95 dB peak SPL). Each neuron was classified according to the effect of the lagging click on its response to the leading click, little effect (minimal), increased the response for most LLIs (enhance), decreased the response for most LLIs (suppress), both increased and decreased the response depending on the LLI (enhance/suppress).

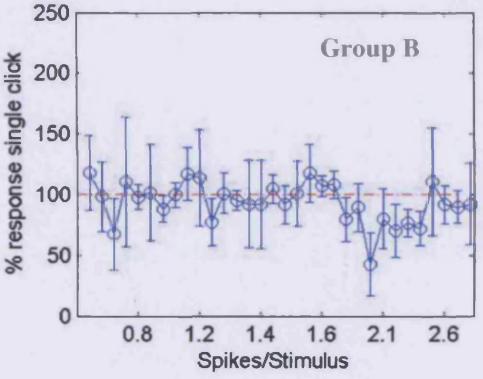
Mean Response to Lead



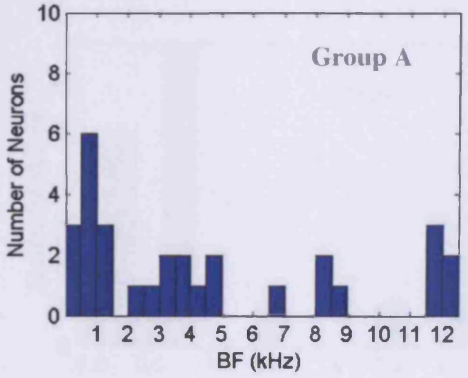
Mean Response to Lead



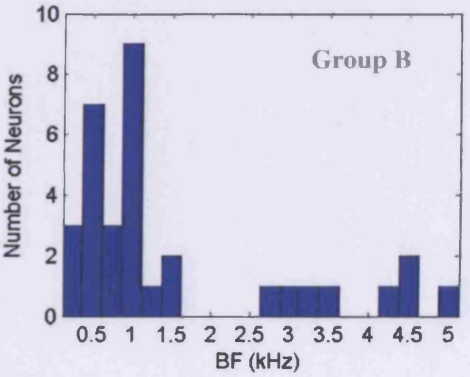




Best Frequency Distribution



Best Frequency Distribution



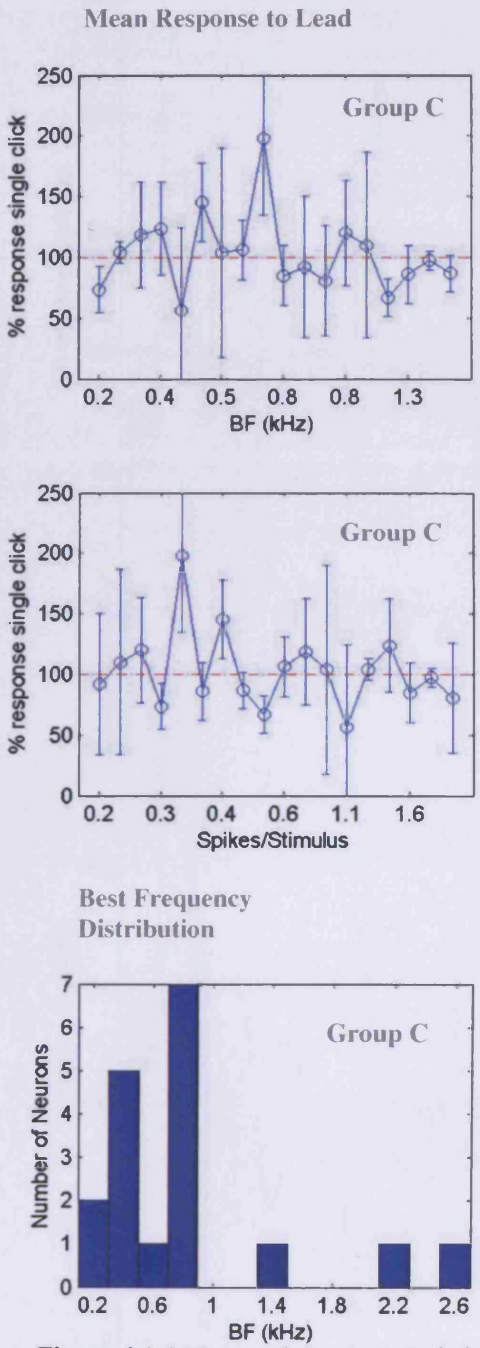


Figure 4.1.6 Mean and standard deviation of percentage change in response to the leading click for three groups of neurons in which the range of LLIs (Group A, 2-20 ms, Groups B, 2-10 ms Group C, 2-5 ms) and intensities (Group A, 40-70 dB peak SPL, Groups B & C, 85-95 dB peak SPL) differed. The top panel in each column of panels is the lead response plotted as a function of BF, the middle panel, lead response as a function of the spikes per stimulus in response to a single binaural click, and the bottom panel the distributions of BFs for each group of neurons.

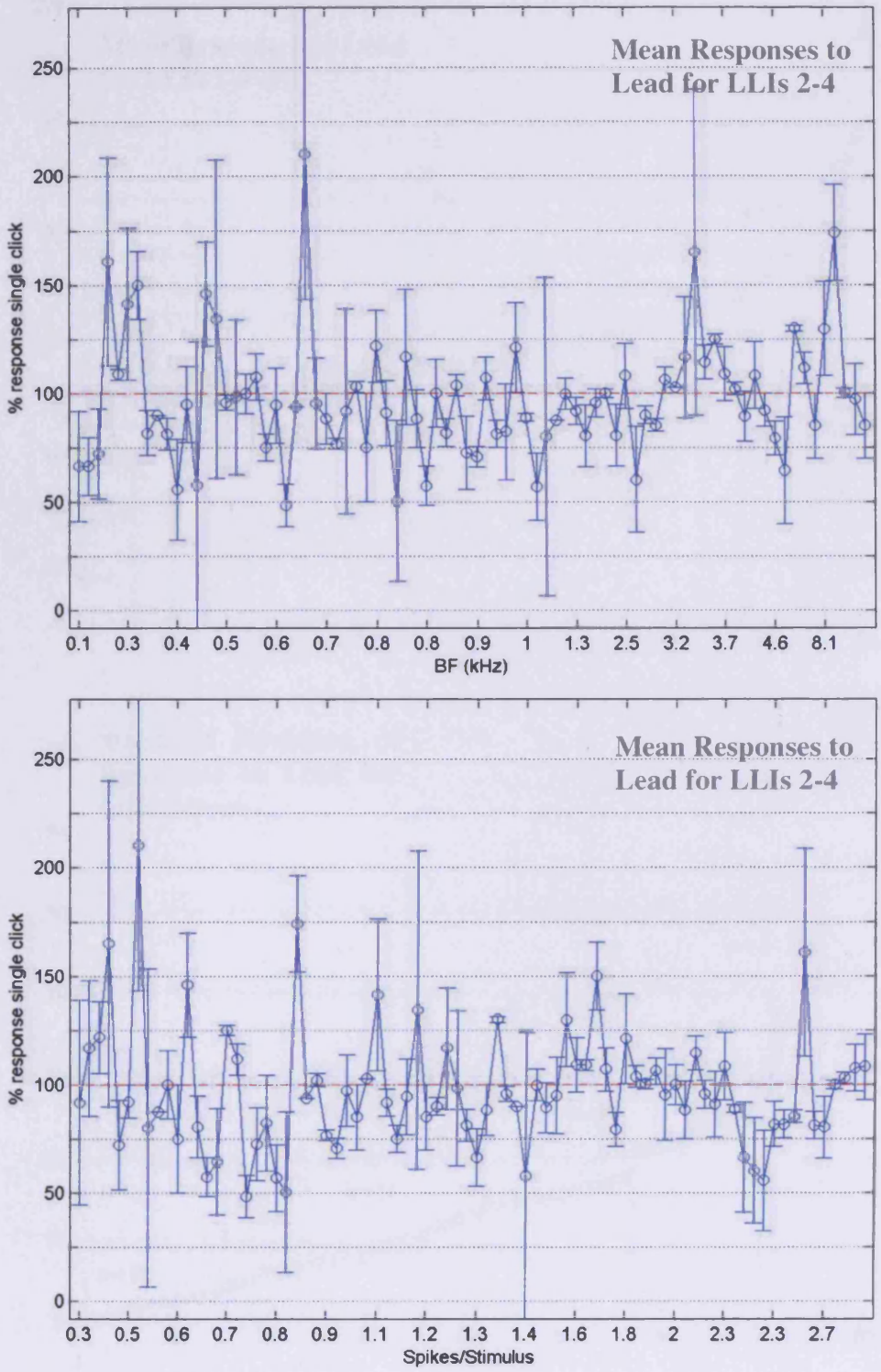


Figure 4.1.7 Mean and standard deviation of percentage change in response to the leading click for LLIs up to 4 ms (N = 80). The top panel is the lead response plotted as a function of BF and the bottom panel the lead response as a function of the spikes per stimulus in response to a single binaural click.

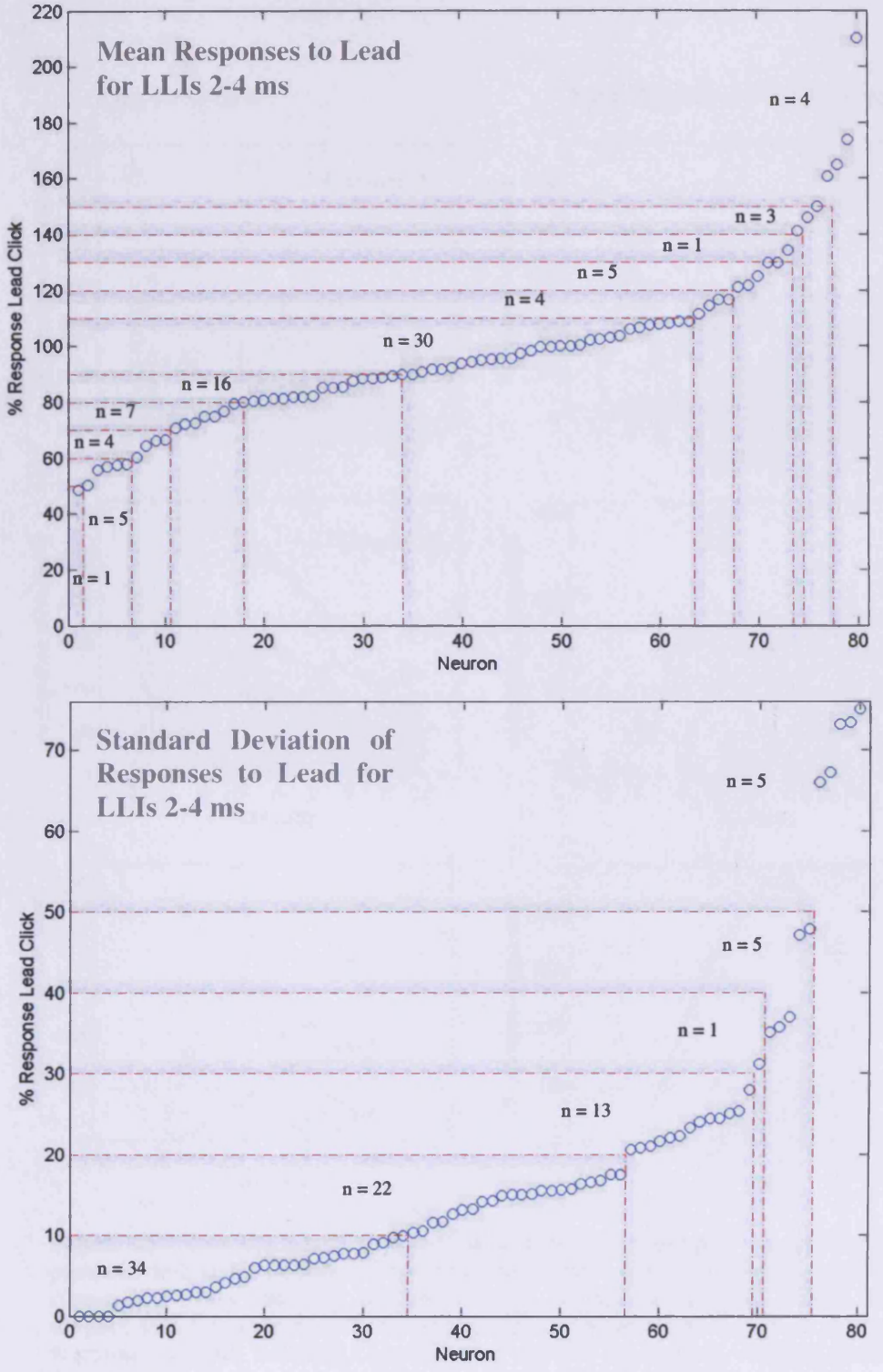


Figure 4.1.8 Mean and standard deviation of percentage change in response to the leading click for LLIs up to 4 ms (N = 80) plotted separately ordered by magnitude. The top is the mean percentage change of the lead response plotted for each neuron and the bottom panel the standard deviation of the response for each neuron. The red lines are plotted for as a visual aid.

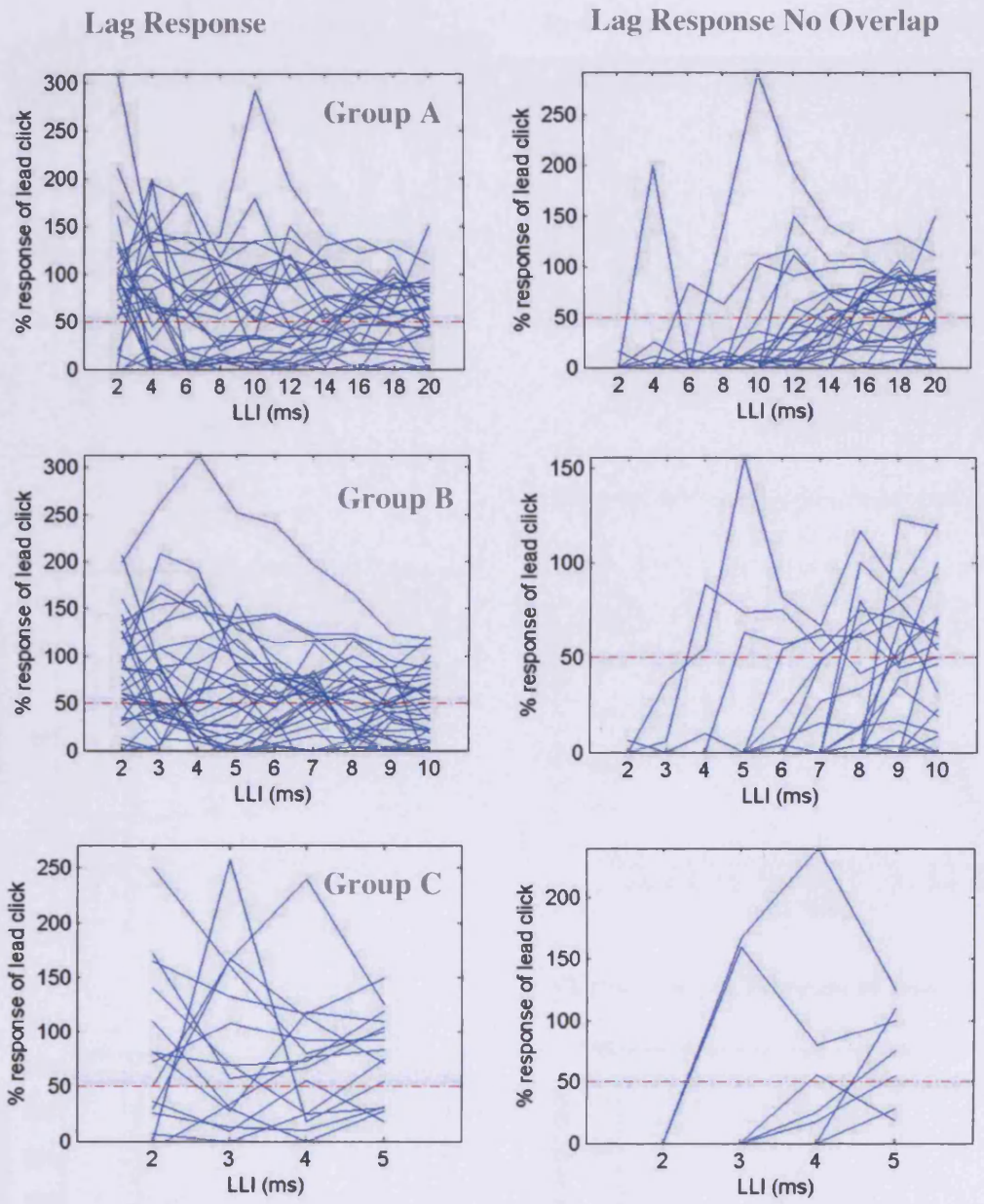


Figure 4.1.9 Recovery function for each neuron from each group of neurons which were presented with single binaural clicks and binaural click pairs with different range of LLIs (Group A, 2-20 ms, Groups B, 2-10 ms Group C, 2-5 ms) and intensities (Group A, 40-70 dB peak SPL, Groups B & C, 85-95 dB peak SPL). The panels on the left are the recovery functions calculated including LLIs for which the lead-lag response windows overlapped, the panels on the right are recovery functions calculated without overlapping response windows.

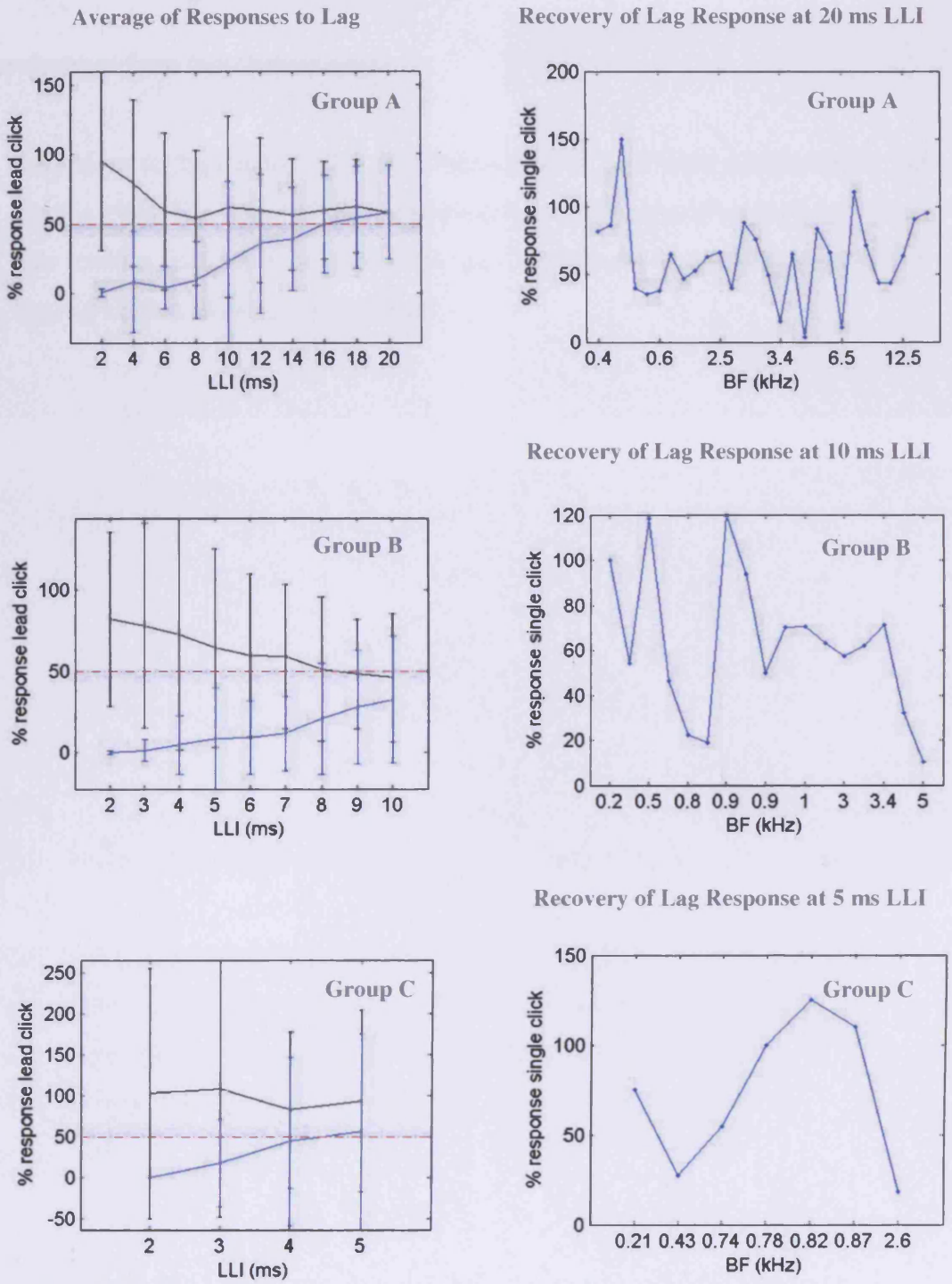


Figure 4.1.10 Average of recovery functions for neurons in each group (left side) calculated including overlapping response windows (black line) and without overlapping response windows (blue line) and recovery at the maximum LLI presented plotted as a function of the neurons BF (right side).

4.2 Results Part IV: Key Findings

The key findings from this chapter were,

1. Responses to the leading click of a binaural click pair were modulated by the lagging click. The effect of the lagging click could be enhancive or suppressive.
2. The leading click of a binaural click pair suppressed neural responses to the lagging click in all neurons examined.

5.0 Results Part III:

Neural Responses of Low-BF ITD Sensitive Neurons to the Leading Click of a Binaural Click Pair

The data presented in this chapter was recorded from twenty low-BF, IC neurons, sensitive to the ITD imposed on a binaural click. The first aim of this experiment was to determine whether neural responses to the leading click of a binaural click pair were modulated by the ITD of the lagging click. The second aim was to assess the effect of stimulus intensity on any observed modulation of neural responses to the leading click of a binaural click pair as a function of the lagging click ITD. Neurons were presented with single binaural clicks and binaural click pairs separated by lead-lag intervals (LLIs) of 2-5 ms. The leading click was presented at the neuron's best ITD, whilst the lagging click was varied from -1.5ms to 1.5ms in increments of 100μs. The stimuli were presented at 3 intensities, 95 dB peak SPL, midway between 95 dB peak SPL and the neuron's response threshold to a single binaural click, and at the neuron's response threshold when presented with a single binaural click. For each neuron, the response to the leading click of a binaural click pair was compared with the response to the same click presented alone, as a function of the ITD of the lagging click, and the LLI of the click pair for each stimulus intensity. Responses to the leading click were calculated by counting the number of spikes occurring from the latency of a neuron's response when presented with a single binaural click, to the time of arrival of the lagging click (see Methods, section 2.5.6). The response to the leading click was compared with the response to a single binaural click, presented with the same ITD and at the same intensity, over the same period.

5.1 The Effect of the Lagging Click ITD on Neural Responses to the Leading Click

For the majority of neurons examined, altering the ITD of the lagging click of a binaural click pair modulated the response to the leading click. The lagging click either enhanced or suppressed neural responses to the leading click depending on its ITD. Additionally, the enhancive or suppressive effect of a given lagging click ITD was dependent on the LLI of the stimulus for some neurons.

Figure 5.1.1 shows the response of an IC neuron (Neuron A; BF = 770 Hz) with two prominent peaks in its ITD function in a series of dot rasters of post stimulus spike times for a selection of lagging click ITDs (best to worst) for LLIs of 2-5ms. For this neuron the best ITD was designated as -0.2ms, the most central peak with a near maximal discharge rate. The red lines indicate the leading click response window. The first line represents the latency of the neurons response to a single binaural click, the second line the time of arrival of the lagging click relative to the neurons response latency. Observation of the leading click response windows indicates the number of spikes evoked in response to the leading click when presented with binaural click pairs was generally suppressed compared to the neurons response to a single binaural click suppressed regardless of the ITD of the lagging click. The degree of suppression in this case was dependent on the LLI of the stimulus with LLIs of 3 and 4 ms appearing more suppressive than 2 and 5 ms. Additionally, the magnitude of the suppression due to the lagging click also depended on the ITD it was presented at for all LLIs. Particularly, the response evoked by the leading click prior to arrival of the lagging click was more suppressed when the lagging click was delivered at unfavourable ITDs (0.6 & 0.4 ms) compared with when it was delivered at favourable ITDs (-0.2 & 0 ms).

Figure 5.1.2 shows the average spike count and standard deviation of neuron A's response to the leading binaural click for the full range of lagging click ITDs plotted in red and the summed response to both pairs of binaural clicks plotted in blue (top 4 panels). The black dotted line indicates the neurons best ITD. Neuron A's response to the binaural click pair (blue line), which did not show a distinct response to the lagging click (see the dot rasters presented in figure 5.1.1), was modulated according to the ITD of the lagging click as indicated by comparison with its ITD function (bottom right

panel, Figure 5.1.1). Further more, the response to the leading click *prior* to arrival of the lagging click (red line) followed the same pattern of modulation indicating neuron A responded to the leading click as if it were responding to an ITD between that indicated by the leading and lagging click ITDs for LLIs of 2-5 ms.

The bottom left panel of Figure 5.1.2 shows the spike count from the leading click normalised to the spike count in response to a single binaural click presented in isolation measured over an equivalent time window (see Figure 5.2.1). Normalised in this way, the responses obtained from the leading click response windows which were of different durations for each LLI, could be compared. The red line shows the response of neuron A to the leading click at 2-ms LLI, the green line 3-ms LLI, the blue line 4-ms LLI and the black line 5-ms LLI for the complete range of lagging click ITDs. For Neuron A the lagging click suppressed the response to the leading click when it was presented at ITDs of -1.5 to 1.5 ms for LLIs of 3-5 ms and both enhanced and suppressed the response at 2-ms LLI depending on its ITD. Regardless of the enhancive or suppressive effect of the LLI, the lagging click was more suppressive when it was presented at ITDs that were more positive or negative than best ITD indicating the neuron responded to a combination of the leading and lagging click ITDs prior to arrival of the lagging click. The degree of modulation of the response as a function of the lagging click ITD was quantified by dividing the minimum number of spikes in response to the leading click by the maximum number of spikes thus taking into account the differences in spike count due to the enhancive or suppressive effects of the LLI. A value of 0 indicates 100% modulation and a value of 1 indicates no modulation. The modulation of the neurons response to the leading click of the binaural click pair was 100% (0), 94% (0.16), 95.5% (0.05), and 71% (0.29) for LLIs of 2-5ms which was greater than that of the neuron's ITD function (74%; 0.36).

Figure 5.1.3 shows the response of a second IC neuron (Neuron B; BF = 420 Hz) with a well-defined best ITD (i.e. the central peak in its ITD function – see top left panel.) and greater modulation of its response with changes to the ITD of a binaural click than neuron A (100% vs. 74%). This neuron's best ITD was designated as -0.2ms, an ITD where the response was maximal. The dot rasters of spike times for each LLI shown in the four bottom panels show when the lagging click ITD was presented at best ITD the response prior to arrival of the lagging click increased relative to conditions where the

lag click was at less favourable ITDs as was the case for the previous neuron (Figure 5.1.1). In this example, however, the effect was more extreme compared to that of the previous neuron, with the complete suppression of the response to the leading click occurring when the lagging click was presented other than at best ITD. For neuron B the effect of the lagging click ITD on the response to the leading click showed greater dependence on the LLI of the stimulus than for neuron A. When the lagging click was presented at best ITD (-0.2ms) and at an LLI of 3ms the response to the leading click was absent, while at an LLI of 5ms the response was similar to the response to a single binaural click. This provides a strong example consistent with phase interactions occurring in the periphery.

As in Figure 5.1.2, the average spike count and standard deviation of the neuron in response to the leading binaural click is plotted in red and the summed response to the both pairs of binaural clicks is plotted in blue in each of the top 4 panels of Figure 5.1.4. The modulation of the spike count in response to the binaural click pair (blue line) of this neuron also followed that of the neuron's ITD function in the bottom right panel as did the response to the leading click prior to arrival of the lagging click (red line). The locations of the peaks in the spike count were not located where both lead and lag clicks evoked the maximum firing rate when presented in isolation (black dashed lines), and were dependent upon the stimulus LLI. As mentioned, in contrast to the example given in Figure 5.1.2 (bottom right panel) the ITD function shown in the bottom right panel of Figure 5.1.4 indicates that the firing rate of the neuron in this example showed greater modulation in response to the ITD of a single binaural click than that of the previous example (100% vs. 74%). The normalised firing rate in response to the leading click displayed in the bottom left panel (Figure 5.1.3) likewise shows 100% (0) modulation for all LLIs in contrast to 100% (0), 94% (0.16), 95.5% (0.05), and 71% (0.29) for LLIs of 2-5 ms for neuron A. For neuron B, the lagging click suppressed the response to the leading click regardless of its ITD for LLIs of 2-4 ms, whereas, for an LLI of 5 ms the lagging click both enhanced and suppressed the response to the leading click. For this neuron the combination of lead-lag ITDs that evoked maximum output was not best (-0.2 ms), best (black dotted line, bottom left panel, Figure 5.1.4) as would be predicted from the response to a single binaural click. Rather, for LLIs of 2, 4 and 5 ms maximum output occurred when the ITD of the lagging click was -0.4 ms and for an LLI of 3 ms when it was -0.3 ms.

Figure 5.1.5 shows the response of a third IC neuron (Neuron C; BF = 450 Hz), this time with an ITD function defined by a trough in the discharge rate (top left panel). For this neuron, best ITD was designated as -0.9ms, an ITD where the response was near maximal. As for the previous two examples presented in Figures 5.1.1 and 5.1.2, when the lagging click was presented at unfavourable ITDs it suppressed the response to the leading click to prior to arrival of the lagging click when compared with the response to either a single binaural click or when the lagging click was presented at best ITD (dot rasters, Figure 5.1.5). Comparison of a particular lag click ITD (e.g. -0.5 ms) across all LLIs reveals that there was also a modulating effect of LLI, as was the case for the previous neurons.

The top 4 panels of Figure 5.1.6 show the average spike count and standard deviation of neuron C in response to the leading binaural click (red line) and the summed response to both pairs of binaural clicks (blue line) for each LLI. The modulation of the spike count in response to the binaural click pair (blue line) followed that of the neuron's ITD function (top left panel) as did its response to the leading click prior to the arrival of the lagging click (red line) as was the case for neurons A and B (Figures 5.1.2 & 5.1.3). Additionally, the locations of the peaks in the spike count, in terms of the ITD of the lag click, were not located where both lead and lag clicks evoked the maximum firing rate when presented in isolation (black dashed lines), and were dependent upon the stimulus LLI. The response to the leading click normalised by the response to a single binaural click is shown in the bottom left panel of Figure 5.1.6. For all LLIs the lagging click had either an enhancive or suppressive effect on the response to the leading click prior to arrival of the lagging click depending on its ITD. The degree of modulation of the response to the leading click prior to arrival of the lagging click as a function of the lagging click ITD was 81% (0.19), 86% (0.14), 85% (0.15) and 87% (0.13) of the maximum response to the leading click for LLIs of 2-5ms (Figure 5.1.6).

Within the sample of 20 neurons 3 different patterns of response to the leading click resulted from varying the ITD of the lagging click. Figure 5.1.7 shows the mean and standard deviation of the responses of 3 neurons (each row of panels) to binaural click pairs (blue line) and the leading click prior to arrival of the lagging click (red line) that capture the observed differences. The black dotted lines on each panel are the neurons

ITD functions. The summed responses (blue curves) to the lead and lag click pairs of the two neurons in the top two rows of Figure 5.1.7 were modulated with the ITD of the lagging click, whereas the summed response of the neuron displayed in the bottom row of panels was essentially flat. The response to the leading click was only modulated in a manner consistent with the ITD function for the neuron presented in the top row of panels, as was the case with the example neurons while the response to the leading click in the other two neurons was flat despite modulation of their ITD functions (black lines).

The modulation of the normalised response to the leading click as a function of the ITD of the lagging click was quantified for the sample of neurons and is presented in Figure 5.1.8. Each bar on the corresponding graph represents the modulation value for each neuron at each LLI. The dotted black line represents the average modulation of the sample of neurons at each LLI. It is apparent from comparison of each LLI that the responses of the majority of neurons to the leading click prior to arrival of the lagging click were modulated by altering the ITD of the lagging click for LLIs of 2-5 ms indicating that this group of neurons responded to an ITD other than that imposed on the leading click. This effect was present for LLIs of 2-5 ms with the average modulation of the spike count for the sample indicating 74% (0.36) modulation of the response to the leading click as a function of the lagging click for 2-ms LLI, 38% (0.42), for 3-ms LLI, 60% (0.40), for 4-ms LLI and 59% (0.41) for 5-ms LLI. The average degree of modulation in response to a single binaural click of varying ITD for the group in isolation was 96% (0.14) (Figure 5.1.9) suggesting the modulation of responses to the leading click while not as significant as that to a single binaural click was still a robust effect extending beyond 5ms LLI.

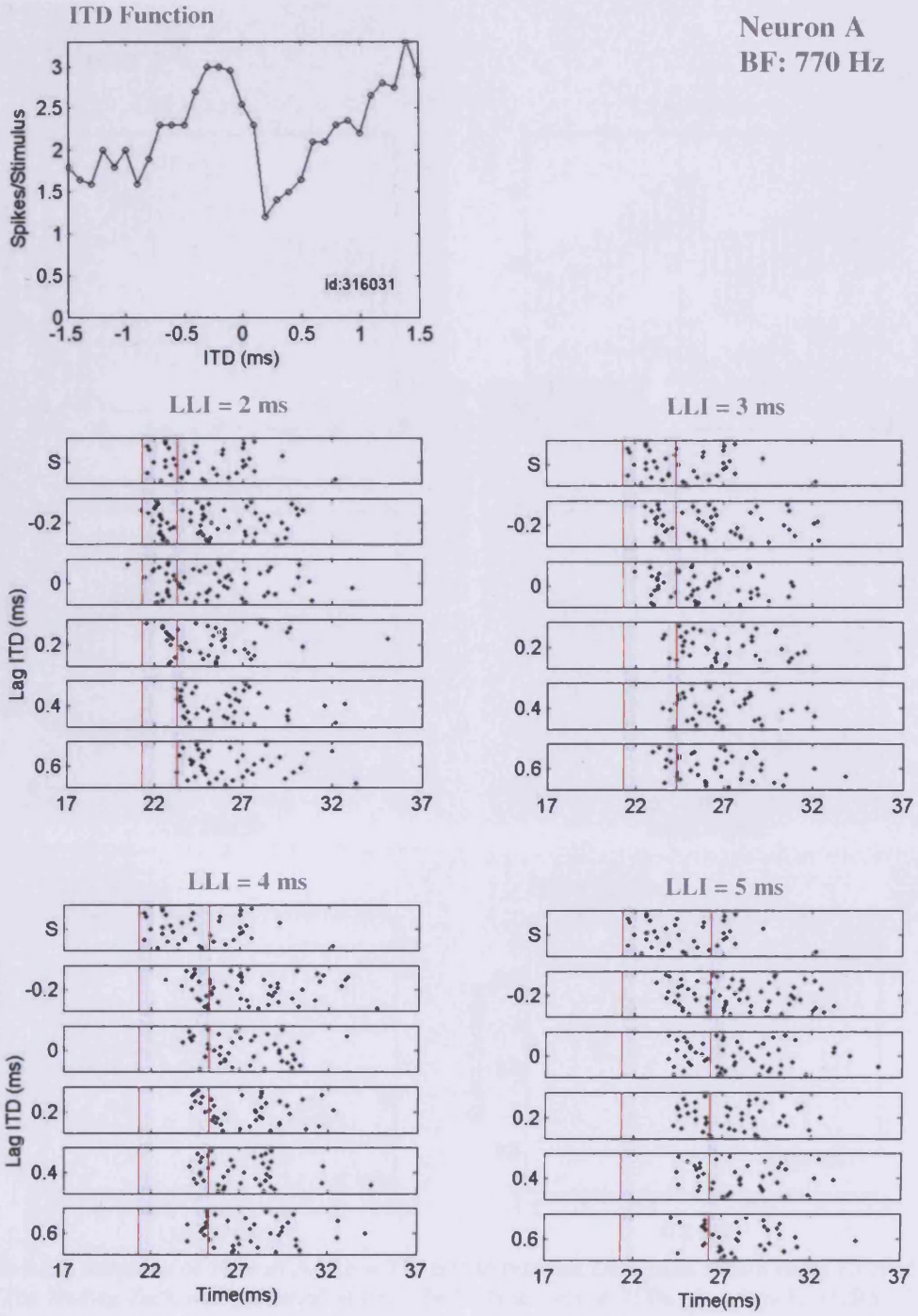


Figure 5.1.1 Dot rasters of post stimulus spikes times in response to a single binaural click at best ITD and binaural click pairs separated by 2-5ms (Bottom 4 panels) for a periodic type ITD sensitive (Top left panel) low BF neuron (770 Hz). The lead click was presented at best ITD, the ITD of the lag click varied from best to worst. Red lines indicate the time window used to calculate the response to the leading click.

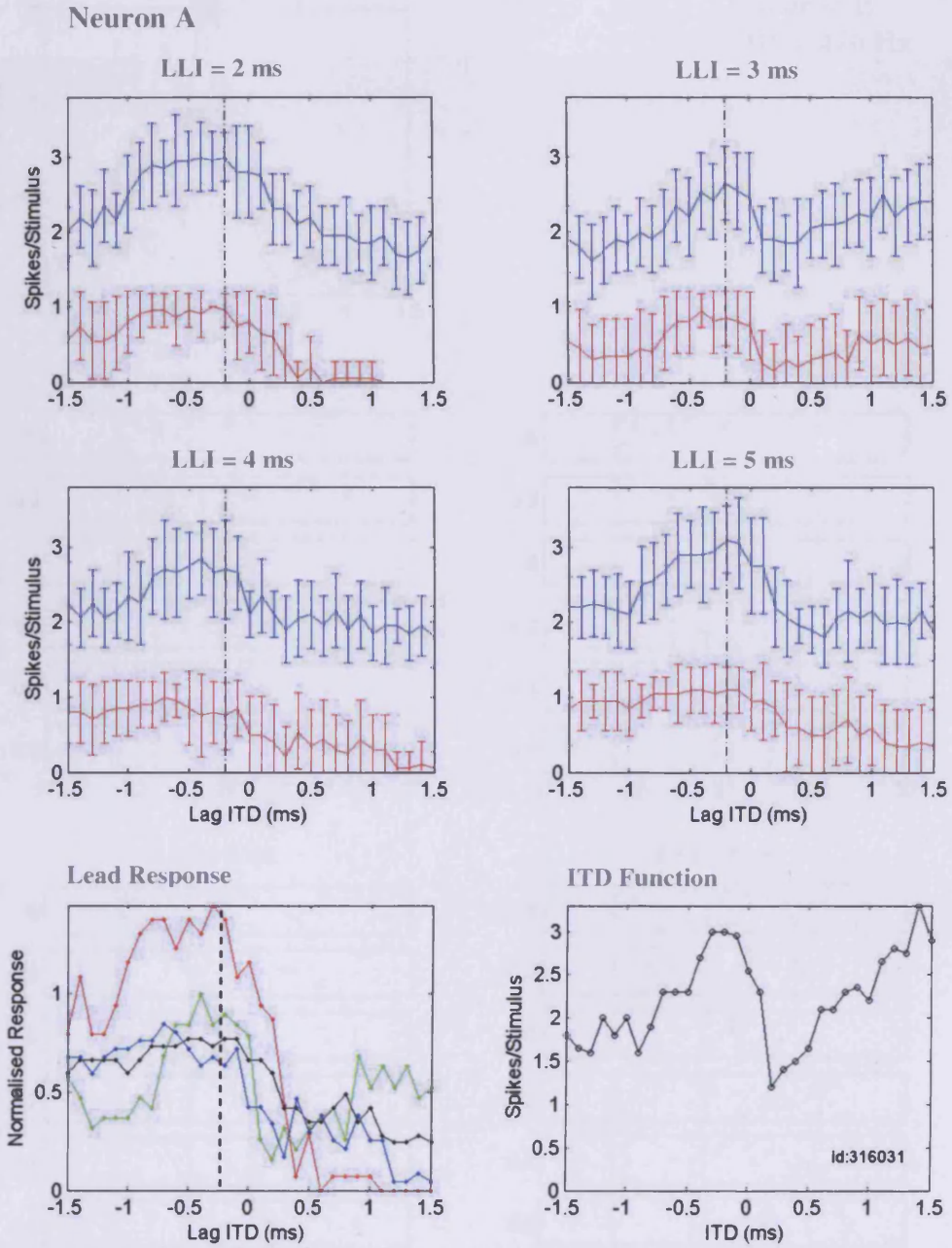


Figure 5.1.2 Response of Neuron A (BF = 770 Hz) to binaural click pairs separated by LLIs of 2-5ms. The leading click was presented at best, the lagging click at ITDs of -1.5ms to +1.5ms. The blue line in the top 4 panels represents the average spike count and standard deviation in response to the leading and lagging clicks, the red line the response to the leading binaural click. The bottom left panel is the average response to the leading click normalised to the average response to a single binaural click over the equivalent time window. The red line represents an LLI of 2ms, green, 3ms, blue 4ms and black 5ms.The bottom right panel is the ITD function of the neuron.

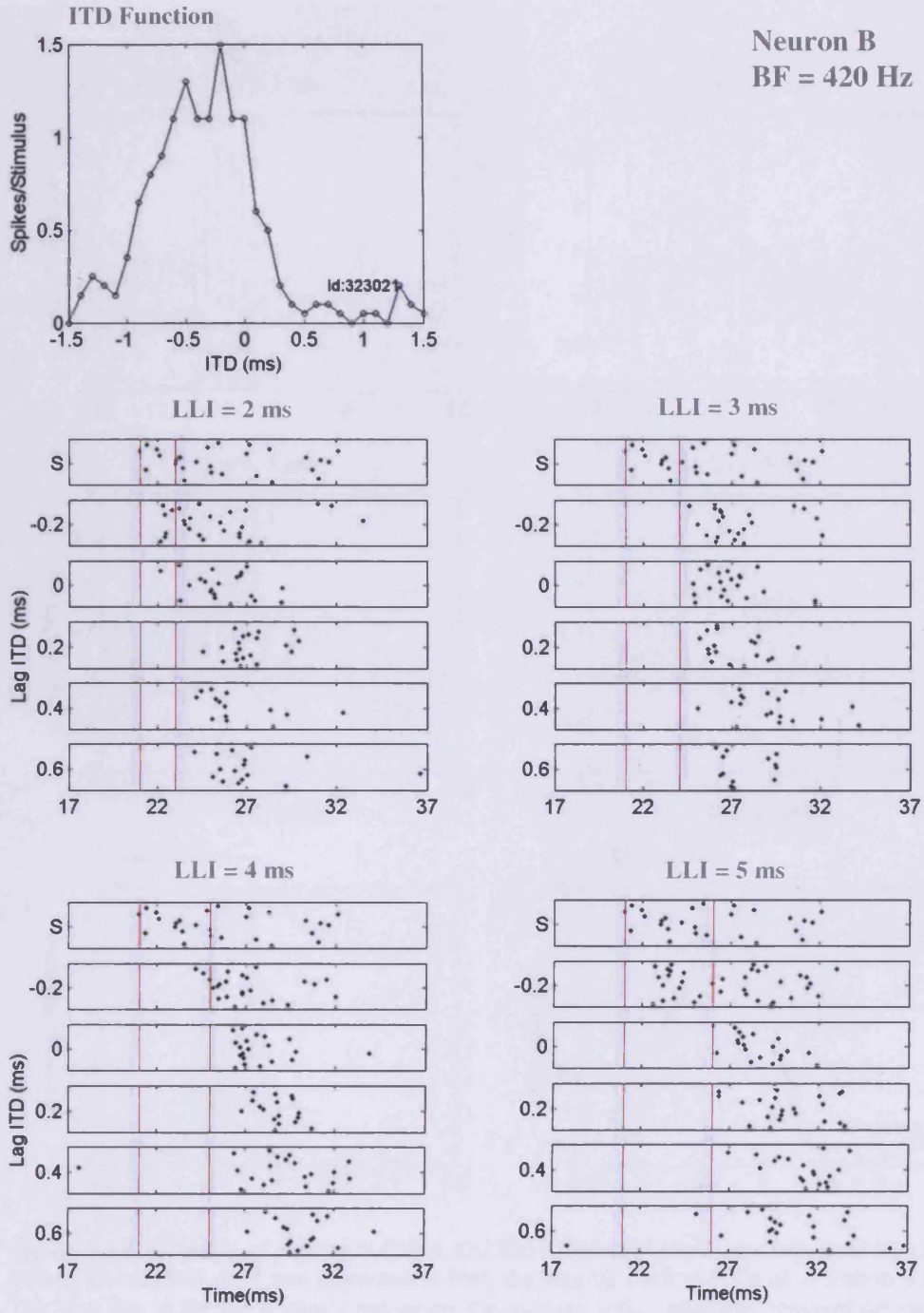


Figure 5.1.3 Dot rasters of post stimulus spikes times in response to a single binaural click at best ITD and binaural click pairs separated by 2-5ms (Bottom 4 panels) for a peak type ITD sensitive (Top left panel) low BF neuron (420 Hz). The lead click was presented at best ITD, the ITD of the lag click varied from best to worst. Red lines indicate the time window used to calculate the response to the leading click.

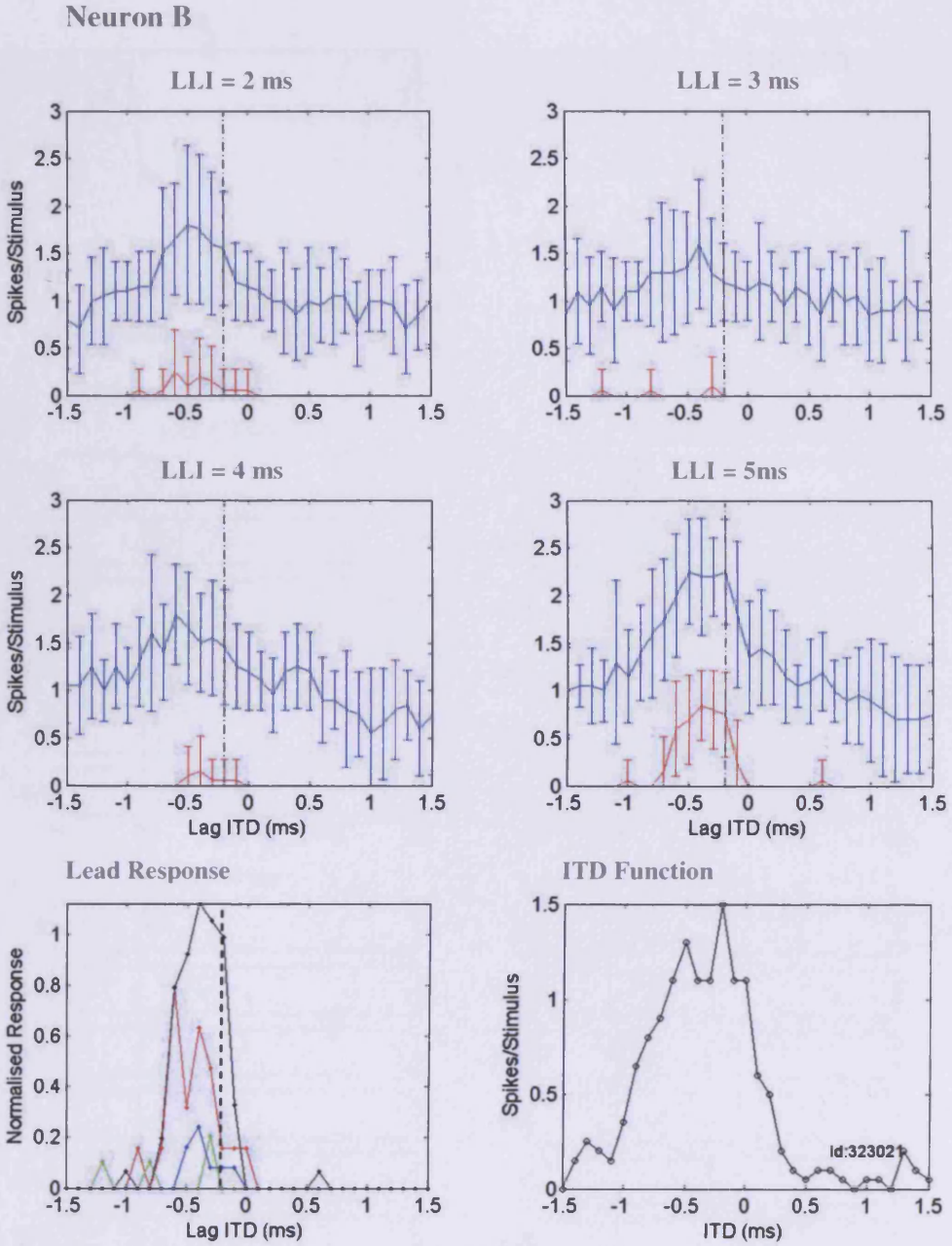


Figure 5.1.4 Response of Neuron B (BF = 420 Hz) to binaural click pairs separated by LLIs of 2-5ms. The leading click was presented at best, the lagging click at ITDs of -1.5ms to +1.5ms. The blue line in the top 4 panels represents the average spike count and standard deviation in response to the leading and lagging clicks, the red line the response to the leading binaural click. The bottom left panel is the average response to the leading click normalised to the average response to a single binaural click over the equivalent time window. The red line represents an LLI of 2ms, green, 3ms, blue 4ms and black 5ms. The bottom right panel is the ITD function of the neuron.

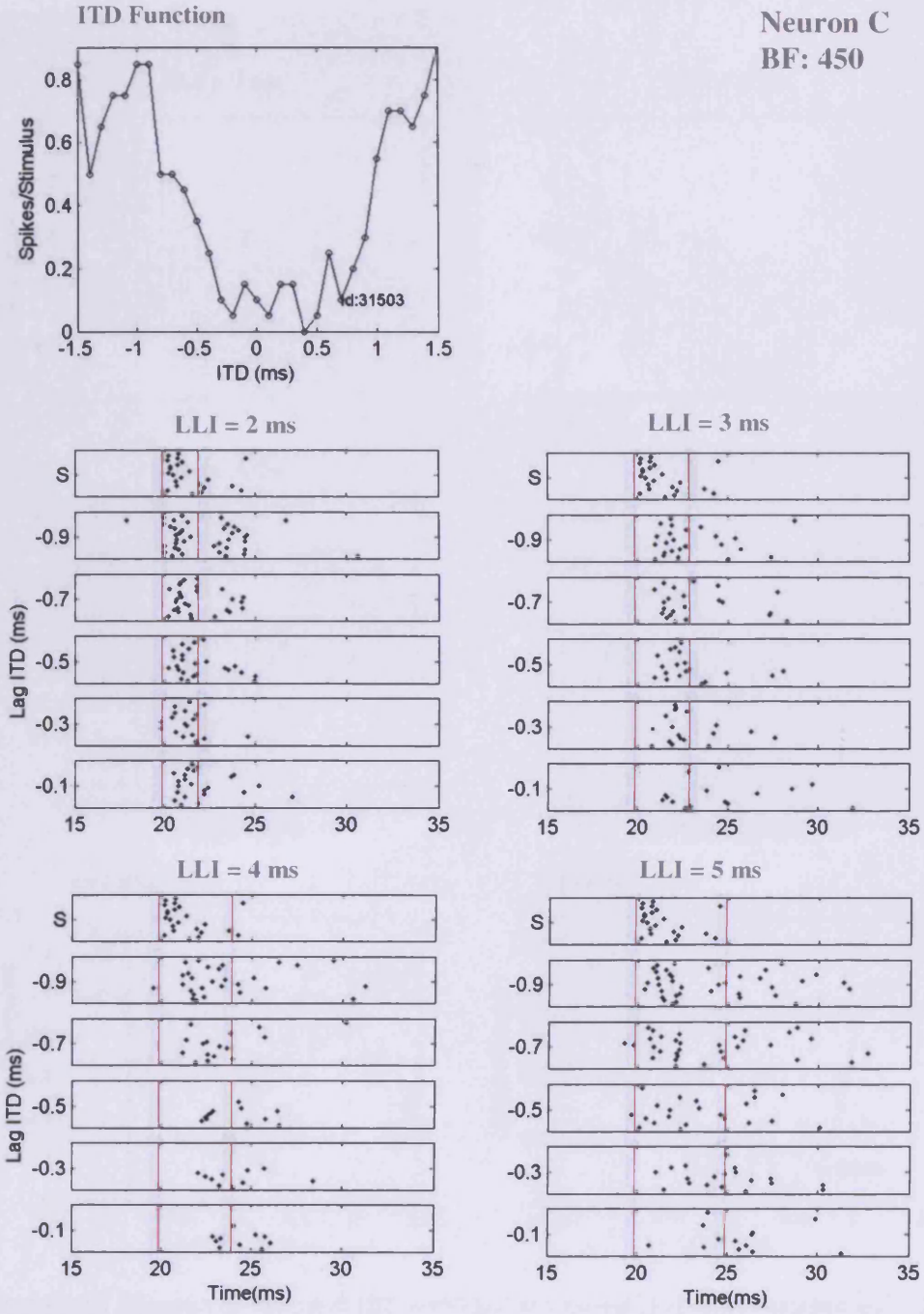


Figure 5.1.5 Dot rasters of post stimulus spikes times in response to a single binaural click at best ITD and binaural click pairs separated by 2-5ms (Bottom 4 panels) for a low BF neuron (420 Hz) with trough type ITD sensitivity. The lead click was presented at best ITD, the ITD of the lag click varied from best to worst. Red lines indicate the time window used to calculate the response to the leading click.

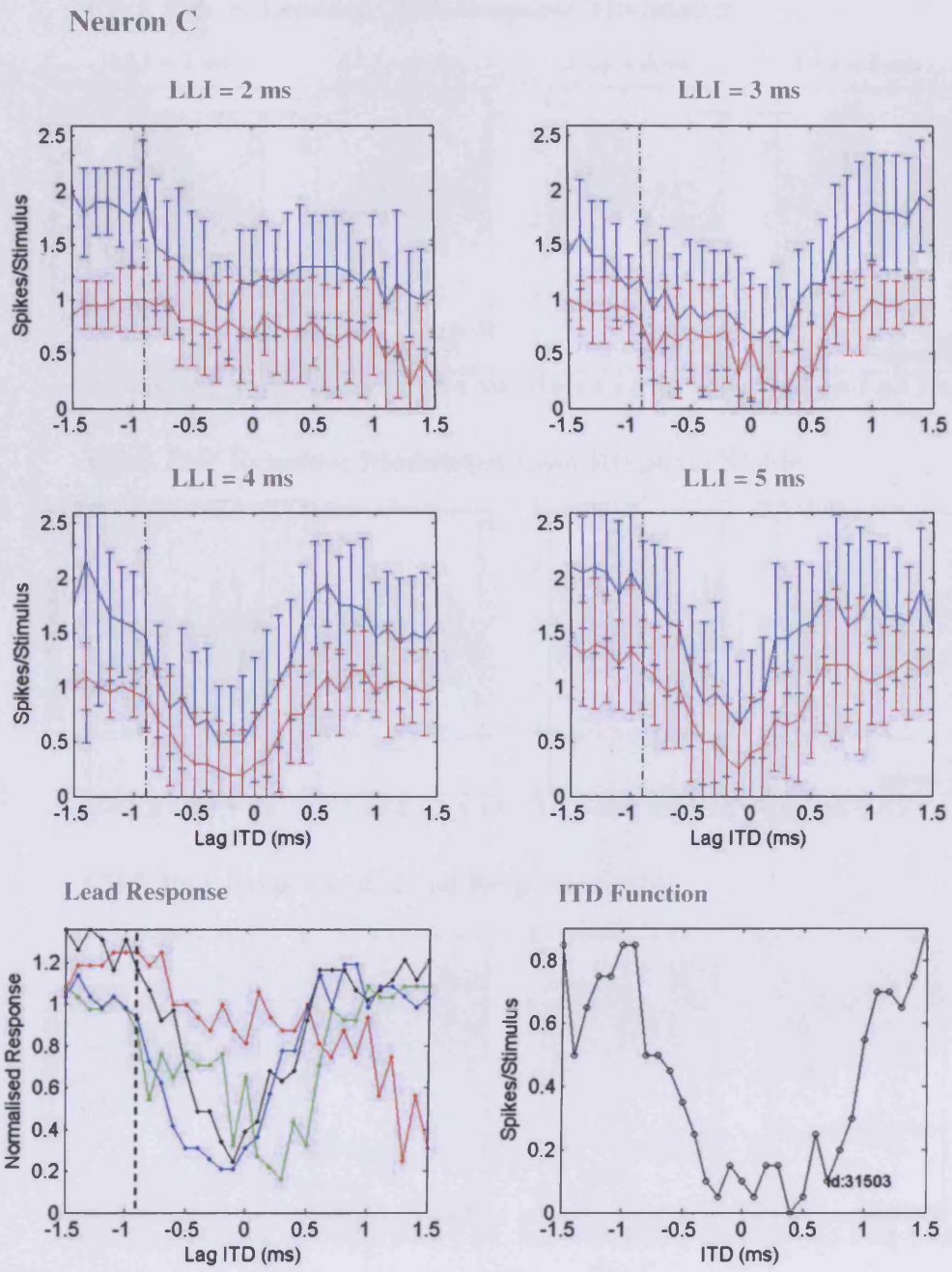


Figure 5.1.6 Response of Neuron C (BF = 450 Hz) to binaural click pairs separated by LLIs of 2-5ms. The leading click was presented at best, the lagging click at ITDs of -1.5ms to +1.5ms. The blue line in the top 4 panels represents the average spike count and standard deviation in response to the leading and lagging clicks, the red line the response to the leading binaural click. The bottom left panel is the average response to the leading click normalised to the average response to a single binaural click over the equivalent time window. The red line represents an LLI of 2ms, green, 3ms, blue 4ms and black 5ms.The bottom right panel is the ITD function of the neuron.

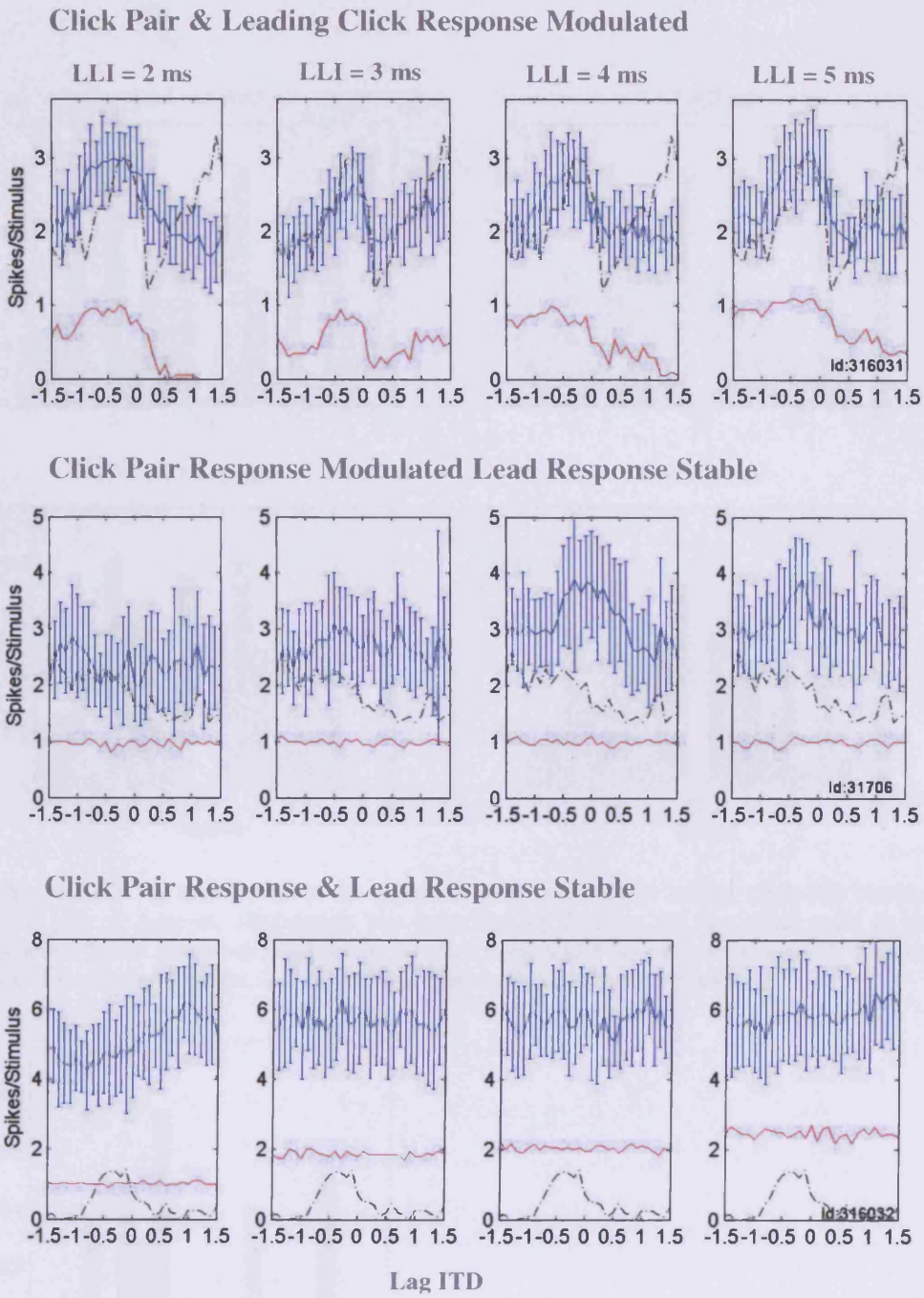


Figure 5.1.7 Variation in types of responses obtained to stimuli where lag click ITD was varied through -1.5-1.5ms in 100μs steps while lead click was held at the neurons best ITD. The LLI was varied from 1-5ms (2-5 shown).The top two neurones exhibited modulation of their response to both the lead and lag click whereas the bottom neurons response was essentially flat (blue line, spike M & SD). The response to the lead click portion of the stimulus, obtained by windowing the spikes occurring from onset to the time of arrival of the lag click (red line), in some cases was also modulated in the same way as the response to the entire stimulus.

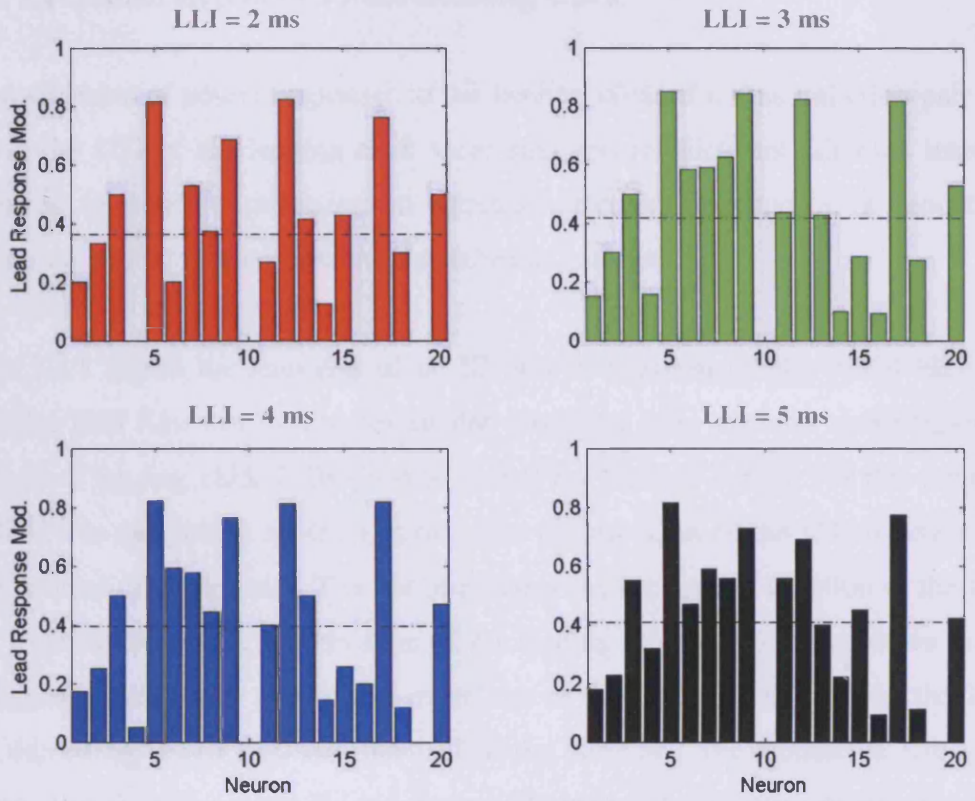


Figure 5.1.8 The modulation of the spike rate in response to the leading click of a binaural click pair all neurons. Modulation was quantified by dividing the maximum point on the function by the minimum point on the function at each LLI for each neuron. Small numbers show greater modulation. The black dotted line represents the group average

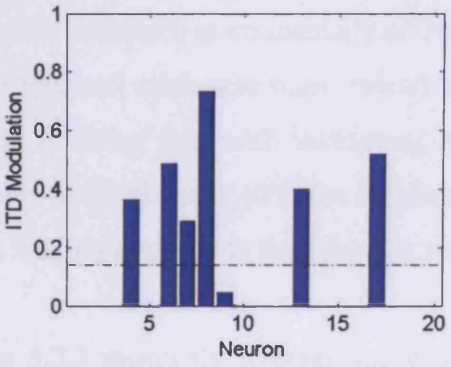


Figure 5.1.9 ITD function modulation for all neurons. The minimum response to a binaural click divided by the maximum response as a function of the stimulus ITD (-4 to +4ms) for all neurons

5.2 The Role of Stimulus Intensity in Mediating the Influence of the Lagging Click on Neural Reponses to the Leading Click

The modulation of neural responses to the leading click of a binaural click pair due to altering the ITD of the lagging click were analysed for different stimulus intensities. For some neurons, a reduction in stimulus intensity resulted in a reduction in modulation while for other neurons it resulted in an increase.

Figure 5.2.1 shows the response of an IC neuron (Neuron D; BF = 800 Hz) with a sigmoidal ITD function in a series of dot rasters of post stimulus spike times for a selection of lagging click ITDs (best to worst) for LLIs of 2-5ms. For this neuron the best ITD was designated as -0.6 ms, the most central point on the ITD function with a near maximal discharge rate. The red lines show the timing and duration of the leading click response windows. Observation of the leading click response windows indicates the lagging click either suppressed or enhanced the neurons response to the leading click depending on its ITD and the LLI of the stimulus. The enhancive effect of the lagging click for this neuron did not necessarily occur when it was presented at an ITD that would have evoked a response from the neuron when presented in isolation (0.6 & 1 ms) as was the case for neurons A-C (section 5.1). Figure 5.2.2 shows dot rasters of the responses of neuron D to the same stimulus conditions as shown in the previous figure but presented at an intensity of 70 dB peak SPL. The response of this neuron to a single binaural click was more robust at the lower intensity, due to a non monotonic change in firing rate with increasing stimulus intensity. In contrast to the response obtained at 95 dB peak SPL the lagging click had little effect on the neurons response to the leading click when the stimulus was presented at 70 dB peak SPL.

Figure 5.2.3 shows the average spike count of the neuron in response to the binaural click pairs and the leading binaural click presented at each stimulus intensity. The blue line and black dashed line represents the average response of the neuron to binaural click pairs separated by LLIs of 2-5 ms for stimulus intensities of 70 dB peak SPL and 95 dB peak SPL respectively. The red line and black dotted line with crosses indicate the responses to the leading click for stimulus intensities of 70 dB peak SPL and 95 dB peak SPL respectively. The response of the neuron to the binaural click pairs was modulated by the ITD of the lagging click for all LLIs at both intensities. Greater

modulation occurred when the stimulus was presented at the higher intensity (black dashed line). Inspection of the corresponding dot rasters (Figure 5.2.1 & 5.2.2) reveals this was primarily due to the less suppressed response to the lagging click when the stimulus was presented at 95 dB peak SPL. The modulation of the response of the neuron to the click pairs was consistent with the neurons ITD function (bottom right panel). The response of neuron D to the leading click (red line & black dotted line) was also modulated by the lagging click for all LLIs at both stimulus intensities although the pattern of modulation was not in accordance with the neurons ITD function. The degree of modulation of the response to the lading click appears equivalent for both stimulus intensities and due to the difference in the neurons firing rate represents a greater proportion of the neurons response at the higher stimulus intensity. Comparison of the normalised response to the leading click for the two intensities (Figure 5.2.3, bottom left panel) shows that the modulation of the response to the leading click as a function of LLI was less in all cases when the stimulus was presented at 70 dB peak SPL (solid lines) than when it was presented at 95 dB peak SPL (dashed lines). The red lines correspond to an LLI of 2ms, green lines 3ms, blue lines 4ms and black lines 5ms. Figure 5.2.4 shows the modulation values as a function of the lagging click ITD for both levels at each LLI. The degree of modulation of the neurons response to the leading click caused by altering the ITD of the lagging click was less when the stimulus was presented at 70 dB peak SPL compared with 95 dB peak SPL for all LLIs. For 2-ms LLI the response to the leading click was 63% less modulated, for 3-ms LLI, 24% less modulated, for 4-ms LLI 39% less modulated, and for 5-ms LLI 35% less modulated as a result of changing the ITD of the lagging click.

Figure 5.2.5 shows the response of a second IC neuron (Neuron E; BF = 260 Hz) to binaural click pairs presented at 95 dB peak SPL for a selection of lagging click ITDs for LLIs of 2-5ms in a series of dot rasters of spike times. The ITD function of the neuron shown in the top left panel indicates the response of the neuron was only slightly modulated by the ITD of a binaural click. As for the previous examples, the start and end of the leading click response windows are plotted in red. The response of this neuron to the leading click was robust for every LLI and only slightly affected by the ITD of the lagging click. Figure 5.2.6 shows the response of the same neuron to the same stimulus presented at 80 dB peak SPL. The response of the neuron to the leading click at this stimulus intensity was generally very weak, and in contrast to its response

to the leading click at 95 dB peak SPL, was dependent on both the ITD and the LLI of the stimulus. The top four panels of Figure 5.2.7 show the average spike count of the neuron in response to the binaural click pairs (blue line) and the leading click (red line). The black dashed line represents average response to both pairs of binaural clicks at the higher intensity while the black crosses represent the average response to the leading click presented at the higher intensity. The modulation of the spike count to the binaural click pair did not follow that of the neuron's ITD function in the bottom left panel, however the degree of modulation relative to the higher level was reduced. The response to the leading click at the higher intensity was the same regardless of the ITD of the lagging click at all LLIs, however the response to the leading click at the lower intensity was modulated by the ITD of the lagging click for all LLIs. Comparison of the normalised response to the leading click for the two intensities (Figure 5.2.7, bottom left panel) shows that at the lower intensity the modulation of the response to the leading click as a function of LLI was greater in all cases, the peak of the function occurring at more favourable ITDs. Figure 5.2.8 shows the modulation values as a function of the lagging click ITD for both intensities at each LLI. Substantial increases in the effect of the lagging click on the neurons response to the leading click were observed when the stimulus was presented at 80 dB peak SPL relative to 95 dB peak SPL for all LLIs of the stimulus. This increase was 76% for an LLI of 2 ms, 82% for an LLI of 3 ms, 82% for an LLI of 4 ms and 77% for an LLI of 5ms.

Figures 5.2.9 and 5.2.10 show the change in the amount of modulation of each neurons (n = 20) response to the leading click due to the ITD of the lagging click, as a function of the stimulus intensity. Figure 5.2.10 shows a reduction of intensity halfway between 95 dB SPL and threshold, Figure 5.2.9 a reduction in intensity to threshold, positive values indicate a reduction in modulation of the spike rate in response to the leading click as a function of the lagging click ITD and negative values an increase. Neuron D is neuron number 16 and neuron E number 17. Both neurons were selected as examples because they represented relatively extreme effects of altering the stimulus intensity. For both reductions in intensity (Figures, 5.2.9 & 5.2.10), more neurons showed an increase in the modulation of their response to the leading click as a function of the lagging click ITD rather than a decrease in the modulation of their response to the leading click as a function of the lagging click ITD. When the stimulus was reduced from 95 dB SPL to midway between this intensity and the neurons threshold in

response to a single binaural click the degree of modulation changed by less than 10% for 8/20 neurons at an LLI of 2 ms, 11/20 neurons at an LLI of 3 ms, 7/20 neurons for an LLI of 4 ms and 6/20 neurons for an LLI of 5 ms. Of these neurons 4/8 showed a greater than 20% change in modulation of their response to the leading click as a function of the lagging click at an LLI of 2 ms, 4/11 neurons at an LLI of 3 ms, 4/7 neurons at an LLI of 4 ms and 3/7 neurons at an LLI of 5ms. These values indicated that for the majority of neurons there was little effect of reducing the stimulus intensity on the modulation of their response to the leading click. From the sample of 20 neurons 12 gave responses to binaural click pairs at a stimulus intensity close to the threshold of their response to a single binaural click that allowed analysis of their responses (see Figure 5.2.10). Reducing the stimulus intensity to near threshold resulted in a greater than 10% change in the modulation of the response of 8/12 neurons at an LLI of 2 ms, 8/12 neurons at an LLI of 3 ms, 7/12 neurons for an LLI of 4 ms and 7/12 neurons for an LLI of 5 ms. Of these neurons 5/8 showed a greater than 20% change in modulation of their response to the leading click as a function of the lagging click at an LLI of 2 ms, 7/8 neurons at an LLI of 3 ms, 7/7 neurons at an LLI of 4 ms and 5/7 neurons at an LLI of 5ms. Reducing the stimulus intensity to close to each neurons threshold in response to a single binaural click resulted in a greater number of neurons exhibiting greater modulation in their response to the leading click as a function of the lagging click compared with the response to stimuli presented at 95 dB peak SPL than reducing it midway. A reduction in stimulus intensity therefore resulted in little change in the degree of modulation in response to the leading click as a function of the lagging click for the majority of neurons. Of the neurons that exhibited changes in the amount of modulation of their responses the vast majority showed an increase in the amount of modulation rather than a decrease as a result of reducing the stimulus intensity.

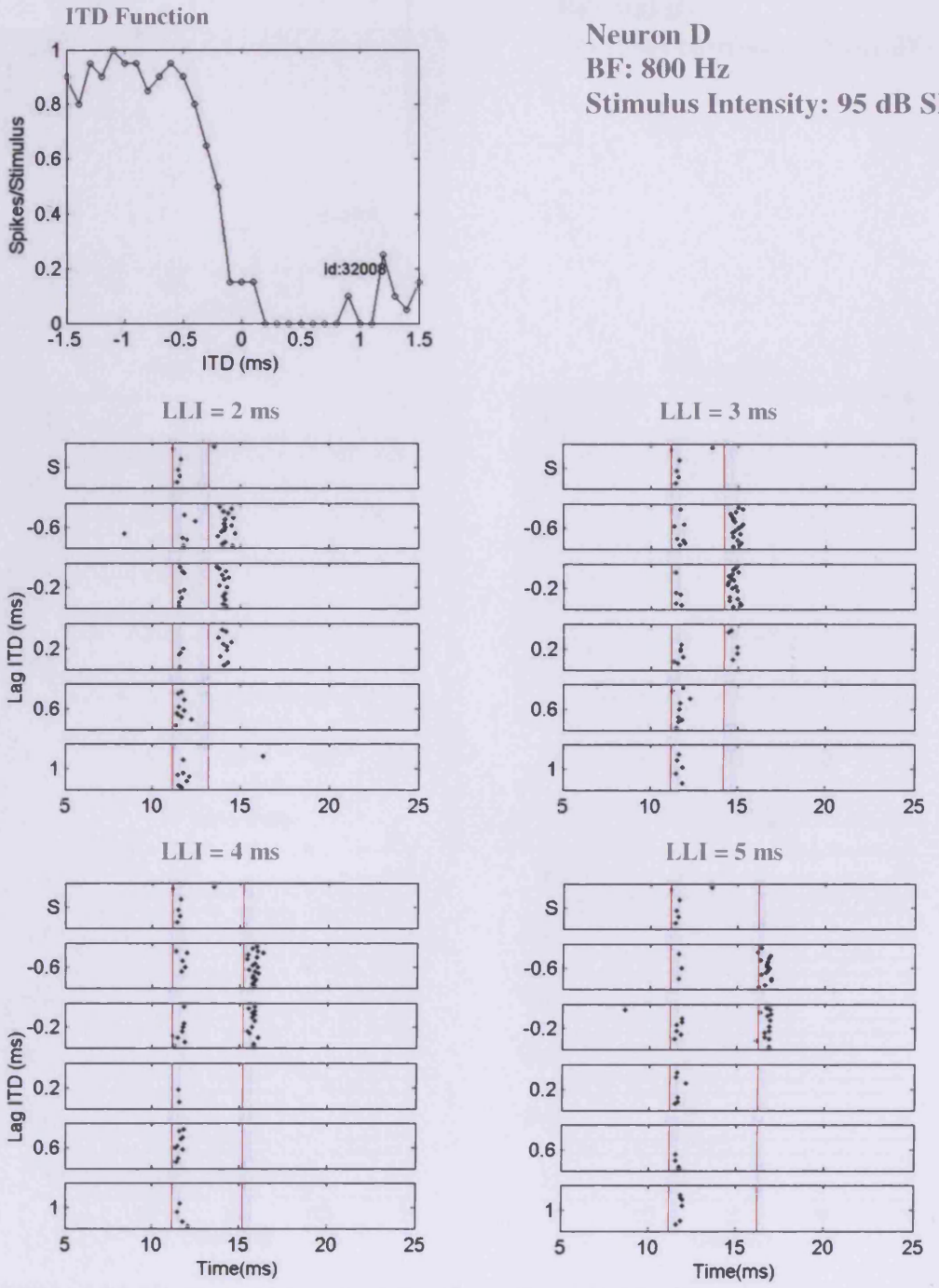


Figure 5.2.1 Dot rasters of post stimulus spikes times in response to a single binaural click at best ITD and binaural click pairs separated by 2-5ms (Bottom 4 panels) for a low BF neuron (800 Hz) with sigmoidal type ITD sensitivity. The level of the stimulus was 95 dB SPL. The lead click was presented at best ITD, the ITD of the lag click varied from best to worst. Red lines indicate the time window used to calculate the response to the leading click.

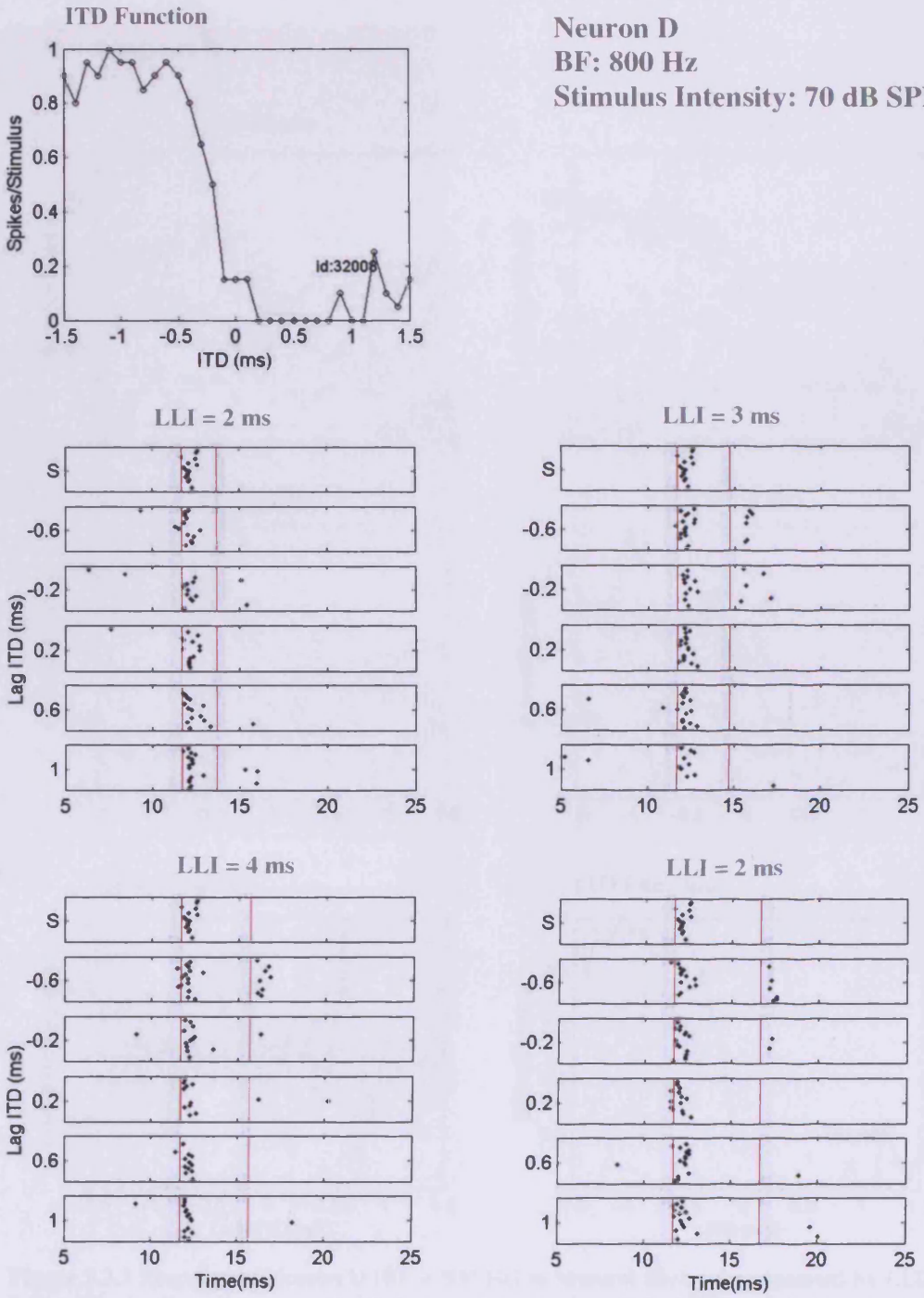


Figure 5.2.2 Dot rasters of post stimulus spikes times in response to a single binaural click at best ITD and binaural click pairs separated by 2-5ms (Bottom 4 panels) for a low BF neuron (800 Hz) with sigmoidal type ITD sensitivity. The level of the stimulus was 70 dB SPL. The lead click was presented at best ITD, the ITD of the lag click varied from best to worst. Red lines indicate the time window used to calculate the response to the leading click.

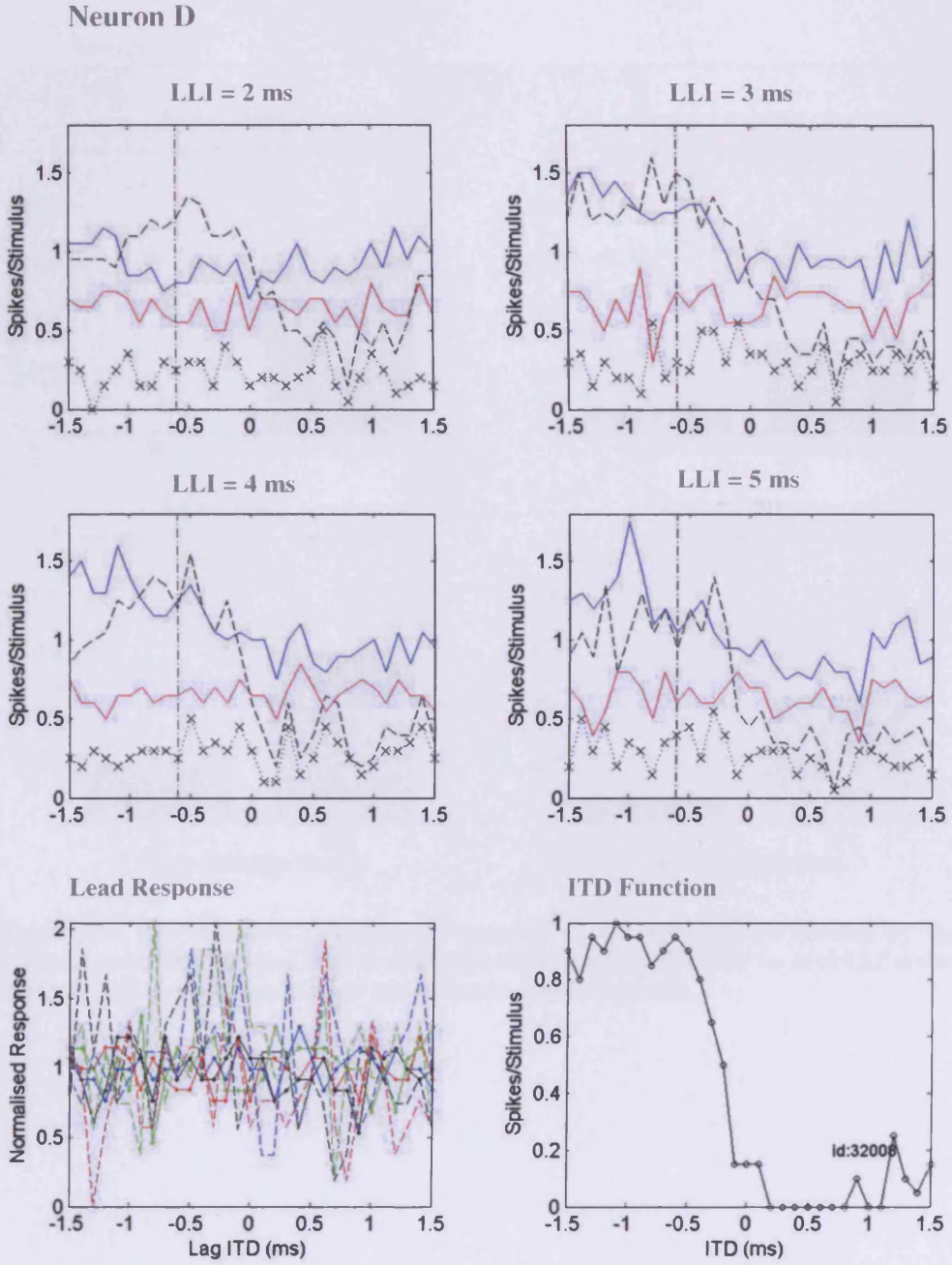


Figure 5.2.3 Response of Neuron D (BF = 800 Hz) to binaural click pairs separated by LLIs of 2-5ms. The leading click was presented at best, the lagging click at ITDs of -1.5ms to +1.5ms. The blue line in the top 4 panels represents the average spike count and standard deviation in response to the leading and lagging clicks, the red line the response to the leading binaural click. The bottom left panel is the average response to the leading click normalised to the average response to a single binaural click over the equivalent time window for each LLI. Solid lines represent a stimulus level of 70 dB SPL, dashed lines 95 dB SPL. The red line represents an LLI of 2ms, green, 3ms, blue 4ms and black 5ms. The bottom right panel is the ITD function of the neuron.

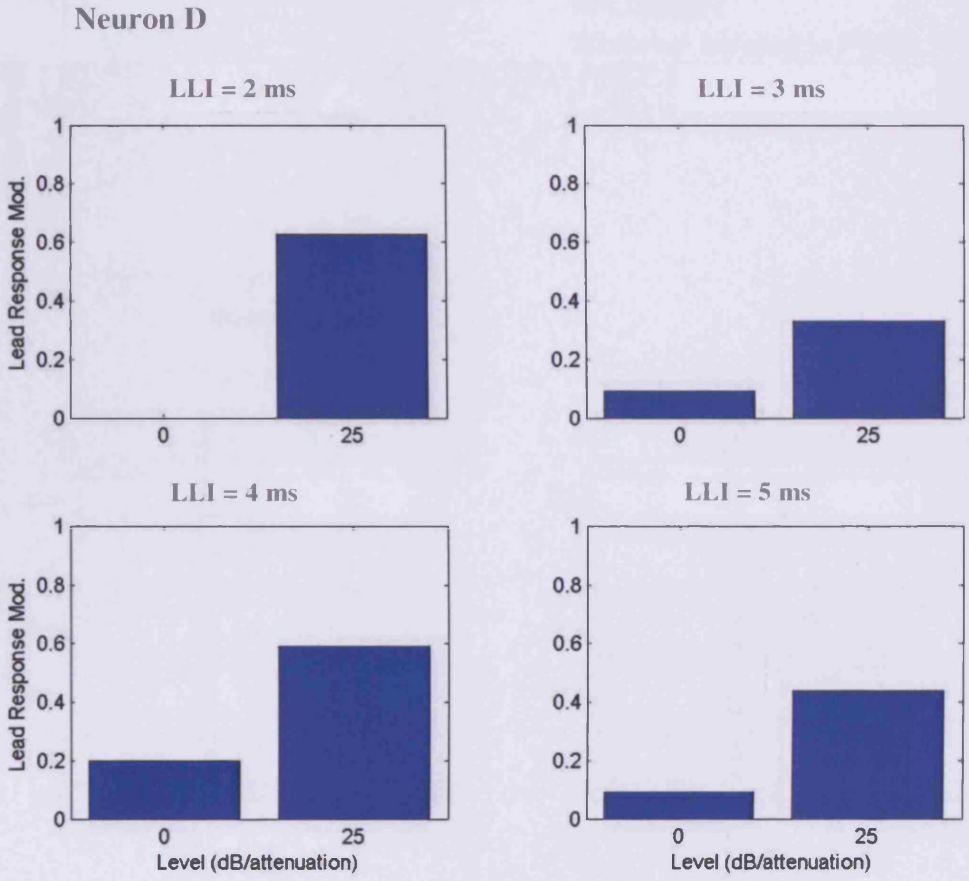


Figure 5.2.4 The minimum response of Neuron D to the leading click divided by the maximum response to leading click as a function of the lagging click ITD for each LLI at two different levels. Low values indicate greater modulation of response.

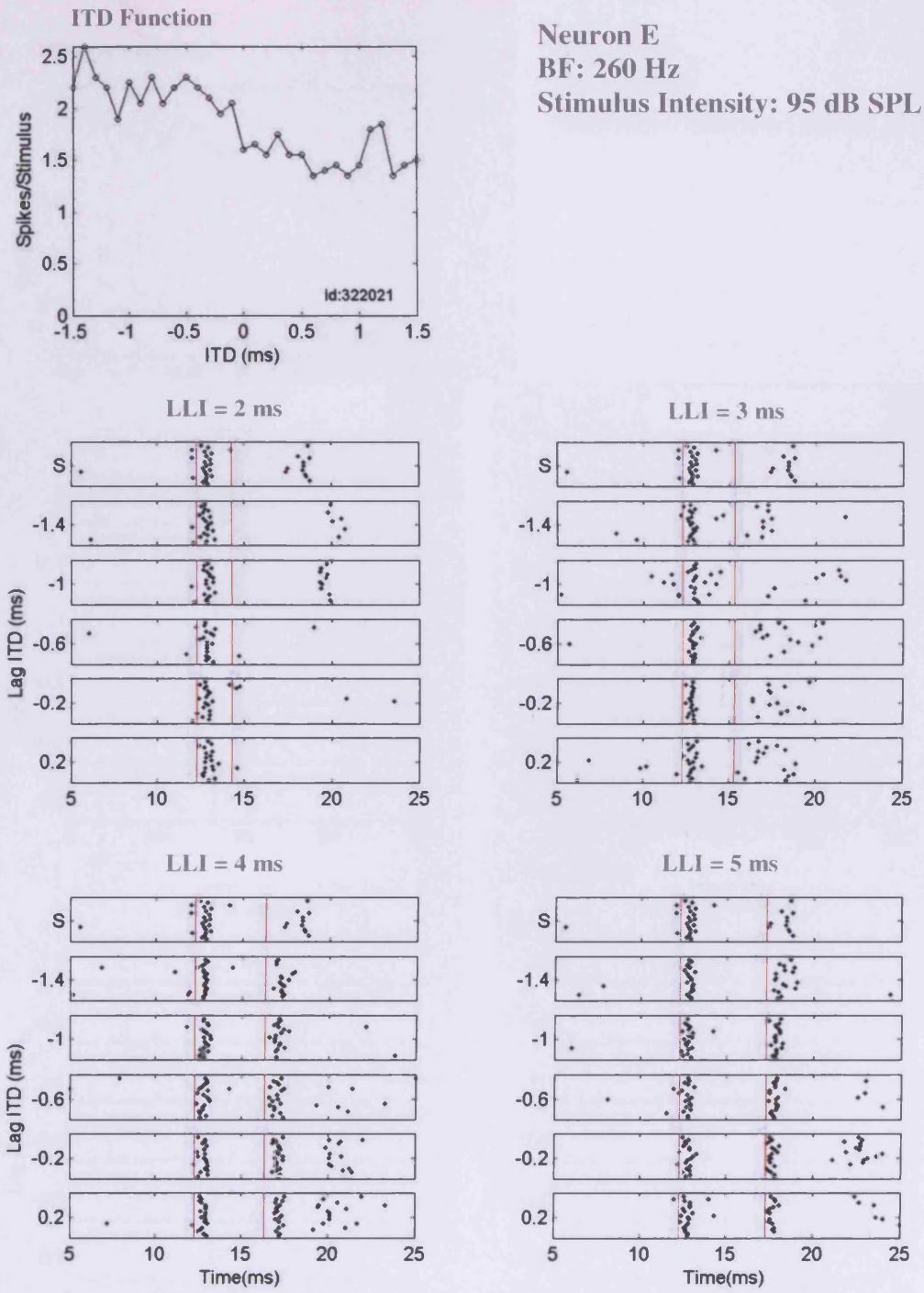


Figure 5.2.5 Dot rasters of post stimulus spikes times in response to a single binaural click at best ITD and binaural click pairs separated by 2-5ms (Bottom 4 panels) for a low BF neuron (260 Hz). The level of the stimulus was 95 dB SPL. The lead click was presented at best ITD, the ITD of the lag click varied from best to worst. Red lines indicate the time window used to calculate the response to the leading click.

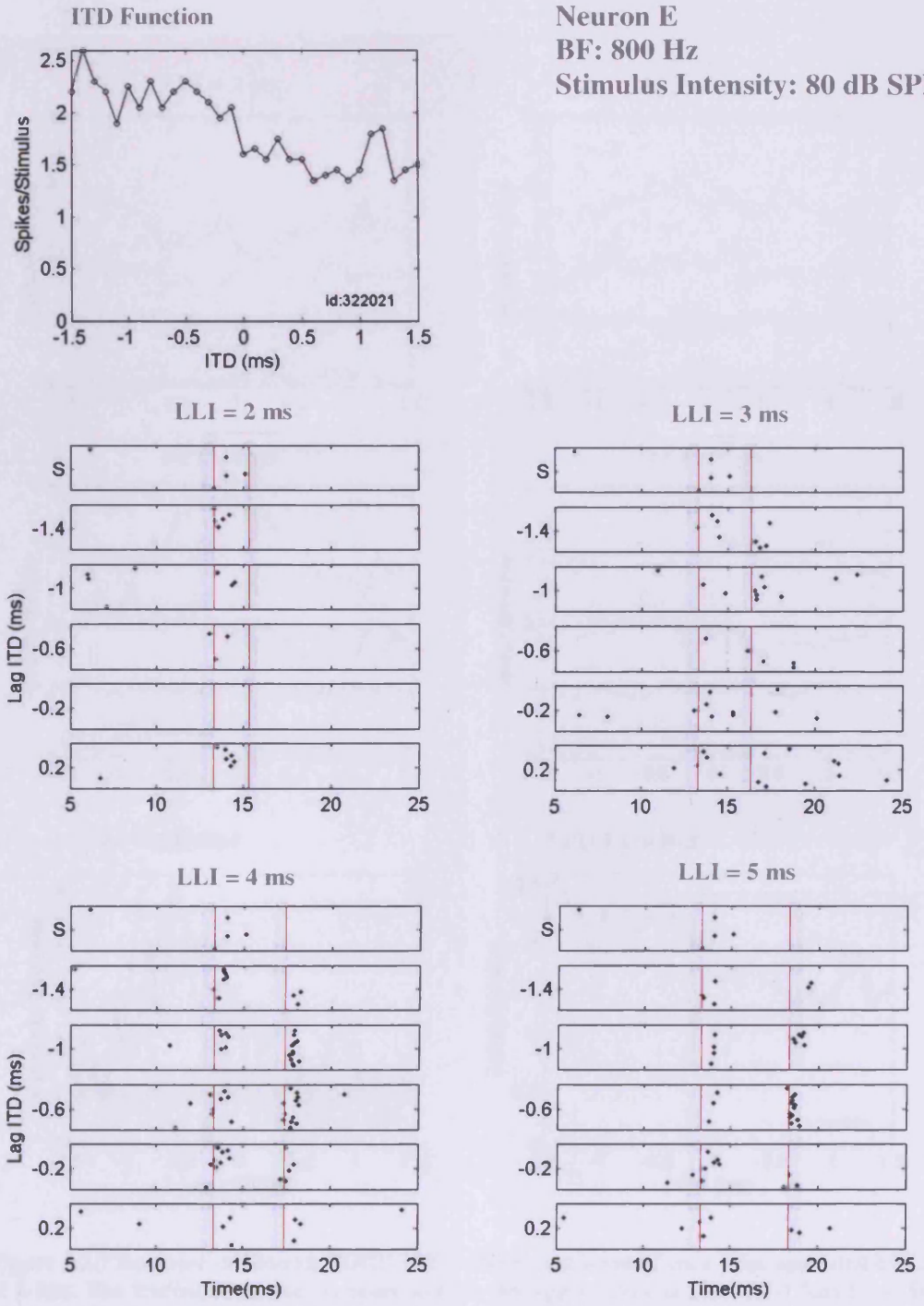


Figure 5.2.6 Dot rasters of post stimulus spikes times in response to a single binaural click at best ITD and binaural click pairs separated by 2-5ms (Bottom 4 panels) for a low BF neuron (260 Hz). The level of the stimulus was 80 dB SPL. The lead click was presented at best ITD, the ITD of the lag click varied from best to worst. Red lines indicate the time window used to calculate the response to the leading click.

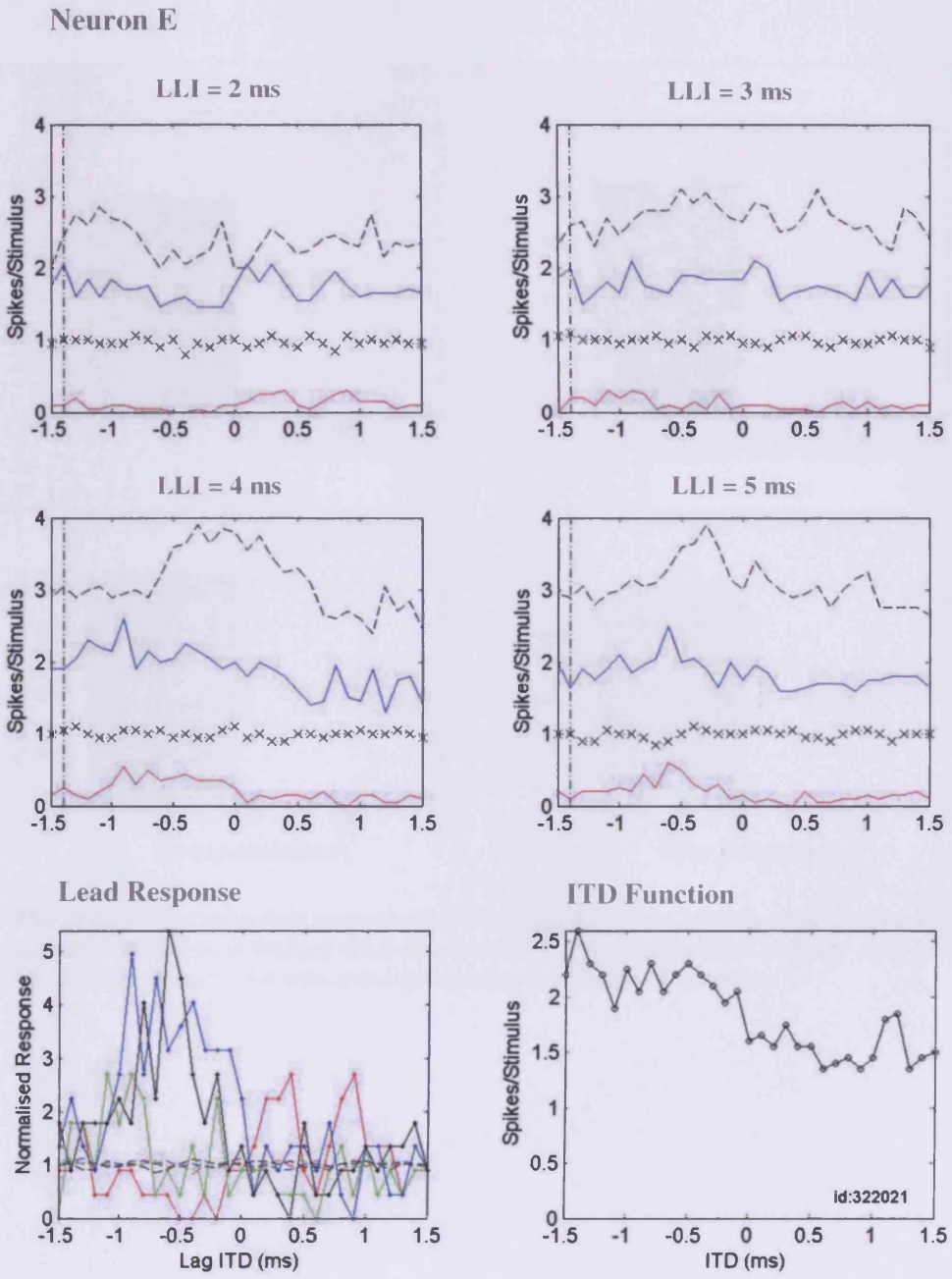


Figure 5.2.7 Response of Neuron 322021 (BF = 260 Hz) to binaural click pairs separated by LLIs of 2-5ms. The leading click was presented at best, the lagging click at ITDs of -1.5ms to +1.5ms. The blue line in the top 4 panels represents the average spike count and standard deviation in response to the leading and lagging clicks, the red line the response to the leading binaural click. The bottom left panel is the average response to the leading click normalised to the average response to a single binaural click over the equivalent time window for each LLI. Solid lines represent a stimulus level of 80 dB SPL, dashed lines 95 dB SPL. The red line represents an LLI of 2ms, green, 3ms, blue 4ms and black 5ms. The bottom right panel is the ITD function of the neuron.

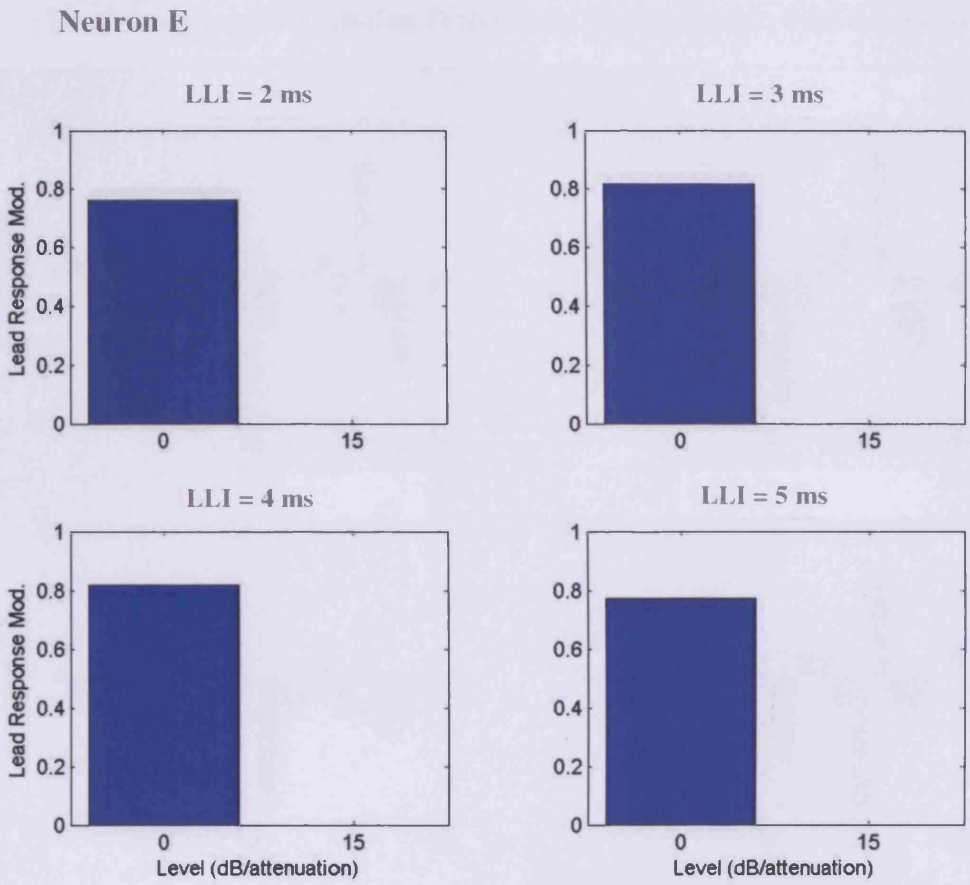


Figure 5.2.8 The minimum response of Neuron 322021 to the leading click divided by the maximum response to leading click as a function of the lagging click ITD for each LLI at two different levels. Low values indicate greater modulation of response.

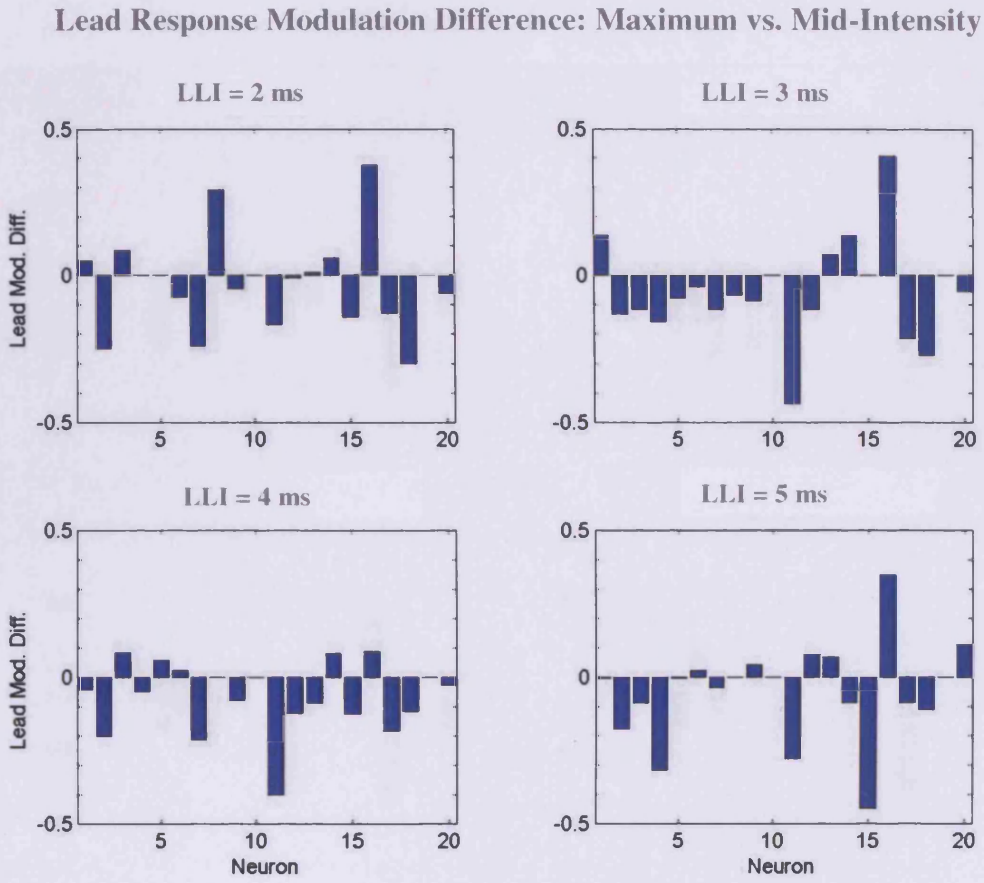


Figure 5.2.9 The difference in the modulation value obtained as a function of lagging click ITD for the highest and intermediate stimulus levels. Negative values indicate an increase in modulation of response, positive values a decrease.

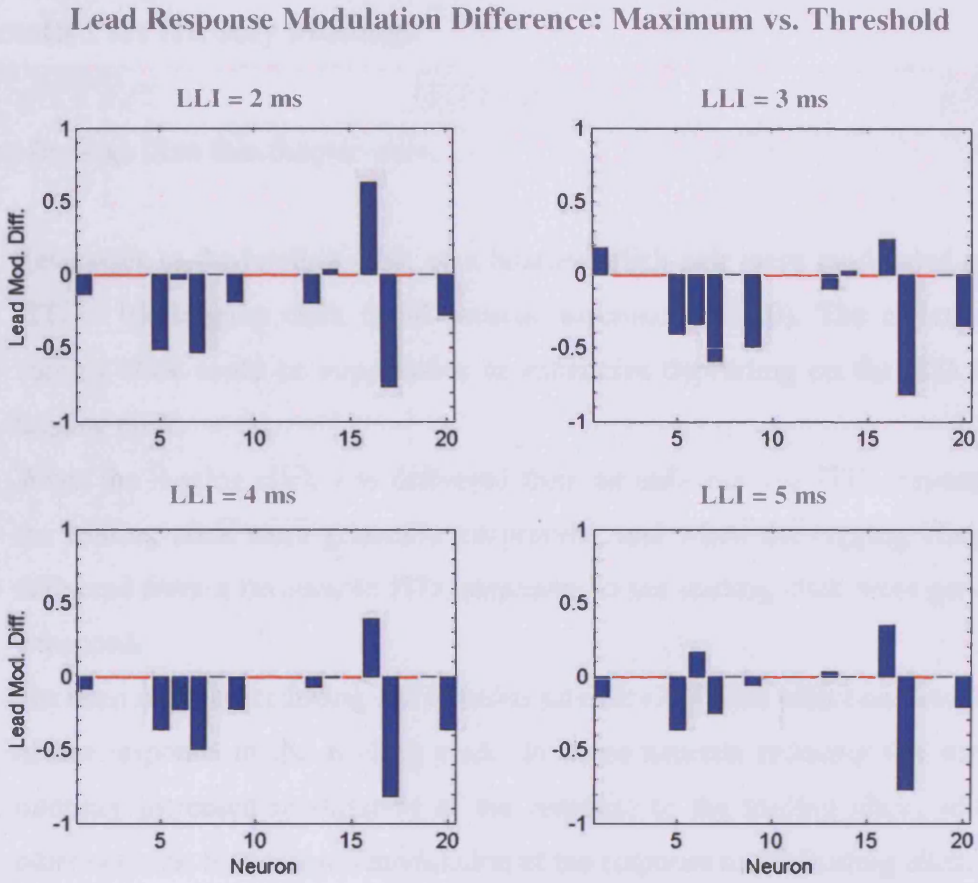


Figure 5.2.10 The difference in the modulation value obtained for each neuron as a function of lagging click ITD for the highest and lowest stimulus levels. Negative values indicate an increase in modulation of response, positive values a decrease. The red lines indicate neurons which had spike rates too low to analyse.

5.3 Results Part III: Key Findings

The key findings from this chapter were,

3. Responses to the leading click of a binaural click pair were modulated by the ITD of the lagging click in all neurons examined (20/20). The effect of the lagging click could be suppressive or enhancive depending on the ITD of the lagging click.
4. When the lagging click was delivered from an unfavourable ITD, responses to the leading click were generally suppressed, and when the lagging click was delivered from a favourable ITD, responses to the leading click were generally enhanced.
5. For most neurons, reducing the stimulus intensity had little effect on modulation of the response to the leading click. In some neurons reducing the stimulus intensity increased modulation of the response to the leading click, while in other neurons, it decreased modulation of the response to the leading click.

6.0 Results Part IV:

The Role of Inhibitory Neurotransmitters in the Neural processing of Binaural Click Pairs

The data presented in this chapter was obtained from 51 neurons located in the central nucleus of the IC of 13 guinea pigs. The aim of this experiment was to determine whether neural inhibition due to the action of γ-aminobutyric acid (GABA) or glycine could account for the neural suppression of responses to the lagging click of a click pair observed in the output of IC neurons. Neurons were presented with single binaural clicks and binaural click pairs separated by LLIs of 2-20 ms. For neurons sensitive to the ITD imposed on a binaural click both the leading and lagging clicks were presented at the neuron's best ITD (Figure 6.0.1), while for neurons *in*sensitive to the ITD of a binaural click stimuli were delivered with an ITD of 0 ms. Stimuli were presented at an intensity approximately halfway between threshold and saturation of the neurons response to a single binaural click. This allowed the response to both the leading and lagging click to increase before saturating. Figure 6.0.2 shows an example of a rate vs. level function for neuron A. The intensity selected for this neuron was 55 dB SPL (red line). Responses to the leading and lagging click were calculated by counting the number of spikes occurring in corresponding time windows (see Methods, section 2.5.6). For each neuron, the responses to the leading and lagging click were compared under normal conditions and after the action of inhibitory neurotransmitters had been blocked by iontophoresis of inhibitory neurotransmitter antagonists.

Figure 6.0.3 shows the distribution of best frequencies (BFs) in the population of neurons (top left panel), half of which were below 2 kHz (26/51), and the other half distributed between 2 kHz and 12 kHz. The population of neurons was comprised of two groups one of which was used to examine the effect of γ-aminobutyric acid (GABA) on neural processing of binaural click pairs (n = 24) and the other to examine the effect of glycine (n = 27). The BFs of the GABA and glycine groups reflected the distribution of the population (top right and bottom panel of Figure 6.0.3). The discharge rates of over half of the neurons sampled (35/51) were modulated by varying the ITD of a binaural click (ITD sensitive). The samples used to examine the effects of

GABA and glycine both contained neurons sensitive to ITDs (12/24 & 23/27 respectively).

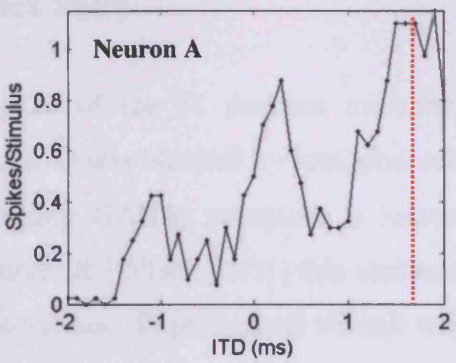


Figure 6.0.1 Average number of spikes in response to a binaural click presented at ITDs of -4 to +4 ms. Red line indicates best ITD.

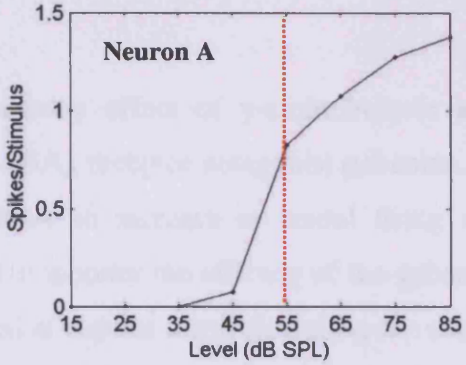


Figure 6.0.2 Average number of spikes in response to a binaural click presented at intensities of 15-85 dB SPL. Red line indicates intensity of stimulus presentation for binaural click pairs.

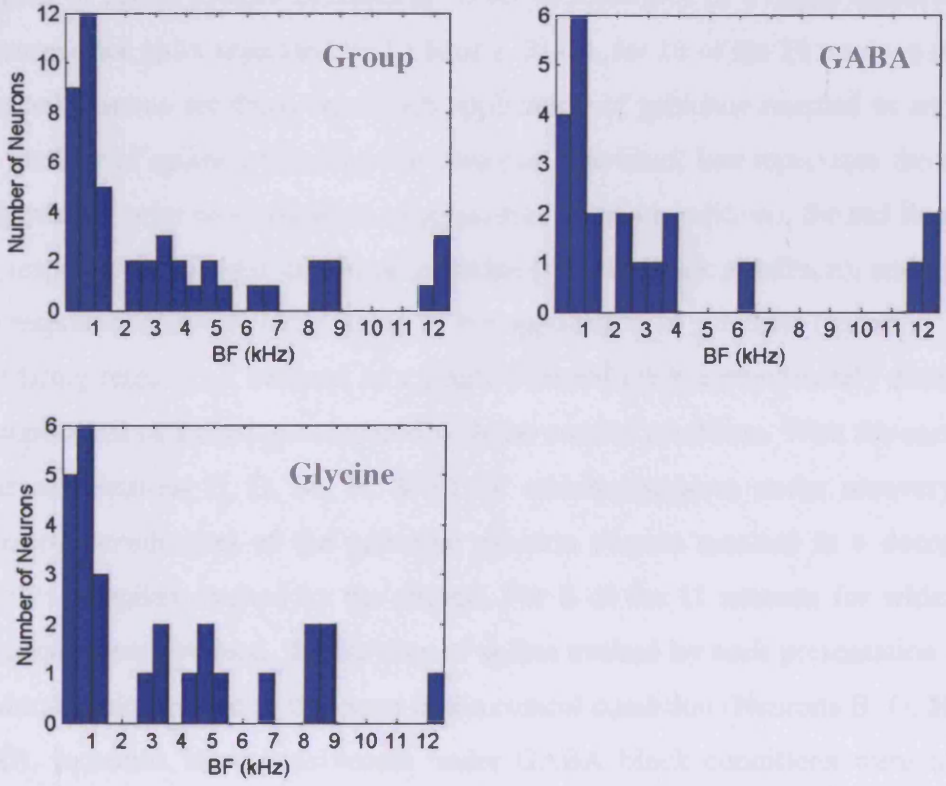


Figure 6.0.3 Histograms of the distribution of best frequencies (BFs) among the sample of neurons. Histograms of BF distribution are shown for the group, and also for those neurons used to examine the effect of γ-aminobutyric acid (GABA) and glycine on neural processing of binaural click pairs.

6.1 The Effect of γ-aminobutyric acid on Neural Processing of Binaural Click Pairs

For 24 of the 51 neurons examined the inhibitory effect of γ-aminobutyric acid (GABA) was blocked by iontophoresis of the $GABA_A$ receptor antagonist gabazine. As blocking $GABA_A$ receptors is known to result in an increase in neural firing rate (Burger & Pollack, 2001) this attribute was used to monitor the efficacy of the gabazine intervention. Experimental stimuli were presented at regular intervals during the course of the drug ejection. When the firing rate of the neuron in response to a single binaural click had approximately doubled, the application of gabazine was terminated and, where possible, the response monitored until it recovered to the firing rate observed prior to drug ejection. Figure 6.1.1 shows the mean and standard deviation of the number of spikes evoked in response to 40 presentations of a single binaural click, and binaural click pairs separated by LLIs of 2-20 ms, for 16 of the 24 neurons studied. The selected neurons are those for which application of gabazine resulted in an increase in the number of spikes evoked by the stimulus. The black line represents the response of each neuron prior to application of gabazine (control condition), the red line represents the response during application of gabazine (GABA block condition), and the blue line the response following termination of the application of gabazine (recovery condition). The firing rates of all neurons to a single binaural click approximately doubled during iontophoresis of gabazine compared with the control condition. With the exception of 5 neurons (Neurons C, D, M, N, & P) for which responses under recovery were not obtained, termination of the gabazine ejection current resulted in a decrease in the number of spikes evoked by the stimuli. For 8 of the 11 neurons for which recovery functions were obtained, the number of spikes evoked by each presentation of a single binaural click returned to that seen in the control condition (Neurons B, G, H, I, J, K, L & O). Increases in responsiveness under GABA block conditions were observed in neurons with low (<2 kHz) and high BFs (>2 kHz).

Three neurons were selected to exemplify changes observed in the neural processing of binaural click pairs when GABA was blocked. For the first two neurons (A & B), blocking GABA resulted in an increase in responsiveness to the leading and lagging click but did not shorten recovery times for responses to the lagging click. Neuron A (BF = 1 kHz) had a short duration response to a single binaural click and, as such,

responses to the leading and lagging click only overlapped at relatively short LLIs. In contrast, neuron B (BF = 0.7 kHz) had a long duration response to a single binaural click and its responses to the leading and lagging clicks overlapped even at relatively long LLIs. For the third neuron (J; BF = 1 kHz), blocking GABA resulted in an increase in responsiveness to the leading and lagging click as well as a shortened recovery time for its response to the lagging click.

6.1.1 Neuron A

Figure 6.1.2 shows the same data as was shown for neuron A in Figure 6.1.1. In the top 11 panels of this figure neuron A's responses are represented as post stimulus time histograms (PSTHs) of the number of spikes evoked in response to a single binaural click and binaural click pairs separated by LLIs of 2-10 ms. The red dotted line on the PSTHs indicates the latency of the neurons response to a single binaural click, and marks the start of the leading click response window. The blue dotted line indicates the arrival of the lagging click relative to the neurons response latency and marks the end of the leading click response window and the start of the lagging click response window. The black dotted line indicates the end of the lagging click response window. The duration of the lagging click response window is equivalent to the duration of the leading click response window. The PSTHs reveal that the neuron responded to binaural click pairs separated by LLIs of 2, 6, 8, and 10 ms with a single continuous response that had one central peak in the spike distribution and was of greater magnitude than the response to a single binaural click. At an LLI of 2 ms there was a shift in the neurons response latency compared with the response to a single binaural click and at an LLI of 4 ms a near absence of a response. At LLIs of 4 and 8 ms, spikes occur after the arrival of the lagging click. However, this response did not appear to be a response to the lagging click, as it was not present for other LLIs, and in both cases was not coincident with the onset of the lagging click. A distinct response to the lagging click, the onset of which was coincident with the arrival of the lagging click, occurred at 12 ms and was present for all subsequent LLIs. The response to the leading click was more robust than that to the lagging click for all LLIs.

The bottom left panel of Figure 6.1.2 shows the number of spikes that occurred during the leading click response window (red line) and the number of spikes that occurred

during the lagging click response window (black dotted line with crosses) for each LLI. The black solid line indicates the number of spikes evoked by the lagging click after the spikes that could be attributed to the neurons response to the leading click had been subtracted (see Methods section 2.5.6). For this neuron, the response to a single binaural click was less than 4 ms in duration and, as such, the leading and lagging response windows captured all the spikes evoked by the stimulus for LLIs greater than 4 ms. Therefore the spike counts derived from each window constitute the neurons response to the leading and the lagging click. For LLIs shorter than 4 ms the response windows do not capture all the spikes evoked by the stimulus, and describe only a portion of the neurons response to the leading and lagging click. Despite this, no distinction is made between overlapping and non-overlapping response windows when describing responses evoked by the leading and lagging clicks.

The bottom left panel of Figure 6.1.2 indicates that the neurons response to the leading click (red line) was greater than the response to the lagging click (black lines) for LLIs of 6-20 ms. For an LLI of 4 ms it indicates the neuron responded minimally to both clicks, and for an LLI of 2 ms the plot indicates the neuron responded to both the leading and lagging clicks. The black dotted line at 2 ms LLI indicates that, without adjustment, the spike count from the lagging click window would have been greater than that from the leading click window despite the response to the leading click being able to account for a number of the spikes. The subsequent reduction in the response to the lagging click at LLIs of 4-10 ms, where all of the spikes were counted, indicates that the increase in the number of spikes occurring during the lagging window for an LLI of 2 ms was not a reliable representation of the lagging click. The increase in the response to the lagging click from 12 ms LLI, and subsequent plateau at 16 ms LLI (black lines), reflects the emergence of the neurons response to the lagging click apparent in the PSTHs.

Figure 6.1.3 shows the responses of neuron A to a single binaural click and binaural click pairs separated by LLIs of 2-20 ms during iontophoresis of gabazine. Iontophoresis of gabazine resulted in an increase in the response to a single binaural click presented at best ITD, from 15 spikes to 38 spikes, indicating the efficacy of the gabazine ejection current. The PSTHs indicate a robust response to the leading click at all LLIs except 4 ms, as was the case in the control condition (Figure 6.1.2), and a

suppressed response to the lagging click occurring from LLIs of 8 ms onward. The responses to the lagging click at LLIs of 4 ms and 8 ms were present in the control condition, although the response at a LLI of 10ms was not. Therefore, the emergence of a consistent response to the lagging click that was present at all subsequent LLIs, occurred 2 ms earlier in the GABA block condition compared with the control condition.

The bottom left panel (Figure 6.1.3) shows the number of spikes occurring during the leading (red solid line) and lagging (black solid line) click windows for LLIs of 2-20 ms. The responses recorded during the leading and lagging click windows in the control condition are represented by the corresponding dotted lines. Comparison of the red lines indicates that the response during the leading click window was greater at all LLIs in the GABA block condition compared with the control condition. Comparison of the black lines shows that while the responses of the neuron in the GABA block condition were different compared with the responses in the control condition, there was not a consistent increase in responsiveness to the lagging click. The spike count during the lagging click window was approximately equivalent in the GABA block condition compared with the control condition for LLIs between 4 ms and 8 ms, after which there was a slight increase in the response until an LLI of 16 ms. At LLIs longer than 16 ms, there was a reduction in the response relative to the control condition. An increase in the lagging click window spike count was also present at an LLI of 2 ms. The increase in the summed spike count in response to the leading and lagging clicks in the GABA block condition compared with the control condition (Figure 6.1.1) could therefore be accounted for primarily by the increased responsiveness to the leading click of the binaural click pair and not by an increase in responsiveness to the lagging click.

The panel on the bottom middle of Figure 6.1.3 shows the difference in the number of spikes evoked by the leading click (red line) and the lagging click (black line) in the GABA block condition compared with the control condition. The neurons responded to the leading click in the GABA block condition with approximately 10 more spikes than in the control condition at all LLIs. The dip in the function at LLIs of 10-14 ms was due to a more robust response to the leading click in the control condition at these LLIs, the increase as a result was relatively lower. The black line indicates that for LLIs where a

distinct response to the lagging click was possible (>4 ms) the neurons response both increased (2, 10, 12, 14 & 16 ms LLI) and decreased (18 & 20 ms LLI). For LLIs where the response to the lagging click increased the increase was proportional to the magnitude of the response observed in the control condition. Spike counts occurring during the lagging click window at these LLIs increased by less than half that seen during the leading click window (approximately 10 spikes vs. 5 spikes) indicating that GABA suppressed the neurons response to the leading click more than it suppressed the neurons response to the lagging click. The increase in the spike count during the lagging click window at an LLI of 2ms appears to have been a result of blocking the inhibition associated with the response to the leading click as the spikes occurring at LLIs of 4-8ms, showed no increase in the GABA block condition compared with the control condition.

The recovery functions calculated for the lagging click in both the control and GABA block conditions are presented in the bottom right panel of Figure 6.1.3 (black and red lines respectively). These were calculated using the leading and lagging response windows and, as such, differ from those presented in the second results section (see Methods section 2.5.6). For this neuron, responses to the lagging click recovered more slowly in the GABA block condition compared with the control condition, with the recovery functions indicating less recovery for LLIs of 14-20 ms in the GABA block condition (red line vs. black line). The decrease in recovery of the neurons response to the lagging click is due to a consistent and robust increase in the neurons response to the leading click and, at best, only a slight increase in responsiveness to the lagging click. Therefore, for this neuron the effect of GABA served to increase the neurons representation of the lagging click rather than suppress it relative to the response to the leading click.

Figure 6.1.4 is presented in the same format as Figure 6.1.3, and shows the response of neuron A to a single binaural click and binaural click pairs separated by LLIs of 2-20ms following termination of the application of gabazine. The PSTHs show the responses to both the leading and lagging click had decreased relative to the drug condition. A total of 23 spikes were counted in response to a single binaural click, higher than the control value of 15. This suggests that the influence of the drug intervention may still have been present. The bottom left panel displaying the response

to the leading click (solid red line) and the response to the lagging click shows that in contrast to the response to a single binaural click the spike counts for the leading and lagging click windows had returned to similar values obtained prior to application to gabazine (dotted lines) for short LLIs, but at longer LLIs the responses to the leading and lagging clicks were reduced relative to the control condition.

6.1.2 Neuron B

Figure 6.1.5 shows the response of neuron B to a single binaural click and binaural click pairs separated by LLIs of 2-20ms. The responses of this neuron are presented in the same format used to describe the responses of neuron A in Figures 6.1.2-6.1.4. In contrast to Neuron A, Neuron B exhibited a longer response to a single binaural click (15 ms vs. 4 ms). As such, it was not possible to observe a discrete response to the lagging click until relatively long LLIs (> 14 ms). The PSTH of the response to a single binaural click shows two prominent peaks in the spike distribution. For LLIs of 2-8 ms, the number of spikes comprising this second peak altered, whilst the location of the peak remained at 25 ms. At an LLI of 8 ms the location of the second peak had not shifted, but corresponded to the time of arrival of the lagging click. For all subsequent LLIs, the second *highest* peak shifted with the LLI of the stimulus corresponding to the arrival of the lagging click. Therefore, for LLIs of 8 ms and greater, a response to the lagging click was apparent although not discrete from the neurons response to the leading click. As the response to the lagging click separated from that to the leading click (LLI $\geq$ 14 ms), the peak observed at 24 ms in the response to a single binaural click again became apparent.

The bottom left panel (Figure 6.1.5) shows the number of spikes counted during the leading click window (red line) and the number counted during the lagging click window (black dotted line) for LLIs of 2-20ms. For this neuron the time windows used to derive the spike counts corresponding to the leading and lagging clicks did not capture the entire response to either the leading or lagging click until an LLI of 14ms. The solid black line represents the spike count in the lagging click window subsequent to adjustment for the portion of the response attributable to the neurons response to the leading click. The unadjusted lagging click spike counts (black dotted line) indicate that the neuron's response to the lagging click was approximately equivalent to its response

to the leading click for LLIs of 4-10 ms. However, as described, a number of these spikes could be accounted for by the neurons response to the leading click (see previous paragraph). Comparison of the leading click spike counts (red line) with the adjusted lagging click spike counts (solid black line) indicates the neuron responded with an equivalent or greater number of spikes to the leading click than to the lagging click for all LLIs. Neither the adjusted nor unadjusted lagging click spike counts describe the neurons spike distribution for LLIs less than 8 ms where the additional spikes, while evoked by the lagging click, did not result in a discernible representation of the lagging click. This case reflects the limitation of using spike counts to describe responses to leading and lagging clicks.

Figure 6.1.6 shows the responses of neuron B to a single binaural click and to pairs of binaural click pairs presented with LLIs of 2-20ms, during iontophoresis of gabazine. The number of spikes evoked in response to a single binaural click more than doubled during iontophoresis of gabazine (35 spikes vs 79 spikes). Neuron B showed a robust response to the leading click at all LLIs. Comparison of the PSTHs representing the neurons response to a single binaural click in the GABA block and control conditions (Figure 6.1.5) shows the neurons response had increased in duration and the cluster of spikes occurring between 20ms and 30ms in the control condition was also evident in the GABA block condition. While this feature is apparent in both conditions for all LLIs where binaural click pairs were presented, in the GABA block condition it is a more robust feature of the spike distribution. As a result a third prominent peak timed with the arrival of the lagging click, representing the neurons response to the lagging click, is not apparent until a LLI of 12 ms in the GABA block condition. In contrast, a response to the lagging click in the control condition was discernable at an LLI of 8 ms.

The bottom left panel of Figure 6.1.6 shows the spike counts that occurred during the leading (red lines) and lagging (black lines) click windows for the control (dotted lines) and GABA block conditions (solid lines). The spike counts indicate that the neurons response to the leading click was more robust than its response to the lagging click for LLIs of 2-14 and 18-20 ms in the GABA block condition. The neuron responded to the leading click with a greater number of spikes for LLIs of >2 ms in the GABA block compared with the control condition (red lines). In contrast, the neurons response to the lagging click for LLIs of <12 ms was reduced in the GABA block compared with the

control condition (black lines). For LLIs of >12 ms, where a representation of the lagging click was apparent in the PSTHs, the neuron responded with a greater number of spikes to the lagging click in the GABA block compared with the control condition. The response during the leading click window therefore accounted for more of the response observed during the lagging click window in the GABA block condition compared with the control condition.

The bottom middle panel of Figure 6.1.6 shows the difference in the number of spikes evoked during the leading (red line) and lagging (black line) click windows in the GABA block condition compared with the control condition. The increase in the number of spikes evoked by the leading click was greater than the increase in the number of spikes evoked by the lagging click in the GABA block condition compared with the control condition for LLIs of 2-14 and 18-20 ms. The spike count during the leading click window increased under conditions of GABA block for LLIs of > 2ms, whereas the spike count during the lagging click window increased for LLIs of 2 and 12-20 ms and decreased for LLIs of 4-10 ms. The decrease in the number of spikes evoked by the lagging click in the GABA block condition was the result of a larger number of spikes occurring during the lagging click response window being attributable to the neurons response to the leading click. As described, the greater increase in responsiveness to the leading click had the effect of reducing the discriminability of the response to the lagging click in the PSTHs in the GABA block compared with the control condition (Figure 6.1.5).

The panel in the bottom right of Figure 6.1.6 shows the lagging click recovery functions for neuron B in the control condition (black line) and GABA block condition. Comparison of the recovery functions indicates that for LLIs < 12 ms the response to the lagging click was more suppressed in the GABA block condition and that for LLIs > than 12 ms recovery was approximately equivalent in both conditions. Therefore, for neuron B, GABA did not selectively suppress the neurons response to the lagging click. For this neuron GABA suppressed the response to the leading click to a greater extent than the response to the lagging click and in doing so made the response to the lagging relatively more suppressed.

Figure 6.1.7, presented in the same format as the previous two figures, shows the response of neuron B to a single binaural click and to binaural click pairs separated by LLIs of 2-20ms following termination of the gabazine ejection current. The PSTHs show the responses to both the leading and lagging click were reduced relative to the GABA block condition (Figure 6.1.6). The spike count in response to a single binaural click had not fully returned to the control value, suggesting some influence of the GABA block intervention was present. The bottom left panel shows the response occurring during the leading click window (solid red line) and the responses during the lagging click window. In contrast to the response to a single binaural click, spike counts from the leading click windows had returned to similar values and were occasionally lower than those obtained prior to iontophoresis of gabazine (dotted red line) for short LLIs, although at longer LLIs spike counts were slightly higher. The spike count during the lagging click window was less than that seen in the control condition at short LLIs but was equivalent at longer LLIs. These values however were closer to the control values than those obtained in the GABA block condition.

6.1.3 Neuron P

Figure 6.1.8 shows the response of neuron P to a single binaural click and binaural click pairs separated by LLIs of 2-20ms. In contrast to neurons A and B, neuron P's response to the lagging click recovered to a greater degree in the GABA block condition (Figure 6.1.9) compared with the control condition. The PSTHs (top panels) show that neuron P responded robustly to the leading click for LLIs of >6 ms. For a LLI of 2 ms there was a single response that was less robust, and that had a longer latency than the response to a single binaural click. A response to the lagging click, that was less robust than that to the leading click, was apparent for LLIs of 4-20 ms. The bottom left panel of Figure 6.1.8 shows the spike counts derived from the leading (red line) and lagging (black line) click response windows for LLIs of 2-20 ms. The spike counts indicate the neurons response to the leading click was greater than to the lagging click for all LLIs. The neurons response to the lagging click began to recover at a LLI of 12 ms and approached the magnitude of the neurons response to the leading click for LLIs of 14-20 ms.

Figure 6.1.9 shows neuron P's responses to a single binaural click and binaural click pairs separated by LLIs of 2-20 ms. The neuron's response to a single binaural click increased from 25 spikes in the control condition to 40 spikes in the GABA block condition indicating the efficacy of the intervention. As was the case in the control condition, neuron P responded robustly to the leading click for LLIs of <6 ms. The response to the leading click was reduced compared with the response to a single binaural click for LLIs of 2 and 4 ms as was the case in the control condition (Figure 6.1.8). However, the latency shift observed for an LLI of 2 ms in the control condition was not apparent in the GABA block condition. Neuron P responded less robustly to the lagging than to leading click for LLIs of 6-20 ms. The bottom left panel of Figure 6.1.9 shows the spike totals derived from the leading (red lines) and lagging (black lines) click response windows for the control (dotted lines) and GABA block condition (solid lines). Neuron P responded to the leading click with a greater number of spikes in the GABA block, compared with the control condition for LLIs of 2-20 ms. The neuron's response to the lagging click was also greater in the GABA block condition than in the control condition for LLIs of 6-20 ms. The bottom middle panel of Figure 6.1.9 shows the difference in the number of spikes evoked by the leading (red line) and lagging (black line) click in the control compared with the GABA block condition. The increase in responsiveness to the leading click was greater than that to the lagging click for LLIs 2-8 and 18-20 ms. However, for LLIs of 10-16 ms neuron P's response to the lagging click increased by an equivalent amount as its response to the leading click in the GABA block compared with the control condition. The bottom right panel of Figure 6.1.9 shows the lagging click recovery functions for neuron P, in the control (black line), and GABA block condition (red line). Neuron P's response to the lagging click recovered by a greater amount in the GABA block condition compared with the control condition for LLIs of 8-12 and 16 ms. The increased responsiveness of the neuron to the lagging click meant that 50 % recovery occurred at an LLI of 10 ms in the GABA block condition compared with 14 ms in the control condition.

Figure 6.1.10, presented in the same format as the previous two figures, shows the response of neuron P to a single binaural click and to binaural click pairs separated by LLIs of 2-20ms following termination of the gabazine ejection current. The PSTH of the neurons response to a single binaural click (top left panel) shows the spike count in response to a single binaural click was 25 spikes, 4 spikes less than in the control

condition, indicating recovery from the effect of gabazine. The PSTHs of responses to binaural click pairs show the neuron responded robustly to the leading click for LLIs of 6-20 ms. Responses to the leading click for LLIs of 2 and 4 ms were less robust than for other LLIs as was the case in the control and GABA block conditions. Neuron P responded to the lagging click for LLIs of 4-20 ms. The weak response to the lagging click at 4 ms LLI was present in both the control and recovery conditions but not in the GABA block condition. The bottom left panel of Figure 6.1.10 shows the spikes counts occurring during the leading click window (red line) and the lagging click window (black line). Neuron P's response to both the leading and lagging clicks has returned to values equivalent to those in the control condition.

6.1.4 Group Results

Figure 6.1.11 shows the spike counts during the leading and lagging click windows in response to binaural click pairs separated by LLIs of 2-20ms for the remaining 13 neurons in which there was an increase in spike counts following application of gabazine. The red lines are the spike counts derived from the leading click window under GABA block (solid line) and control (dotted line) conditions, and the black lines are the spike counts derived from the lagging click window under GABA block (solid line) and control (dotted line) conditions. Comparison of the responses to the leading clicks (red lines) indicates that neurons E-O responded to the leading click with an equivalent, or greater, number of spikes in the GABA block condition compared with the control condition for all LLIs (red dotted line & red solid line). For neurons C and D, the response to the leading click was less for particular LLIs under GABA block (neuron C 4, 6, &10 ms LLI; neuron D 2 ms LLI) but was greater for most LLIs compared with the control condition. With the exception of neurons J, I, and O the neurons response to the lagging click in the GABA block condition (black solid line) was also either equivalent to, or greater than, the response to the lagging click in the control condition (black dotted line). In contrast, neurons J, I, and O also showed decreases in responsiveness to the lagging click in the GABA block condition compared with the control condition. Despite increases in responsiveness to the lagging click in the GABA block condition (black solid line) compared with the control condition (black dotted line) for neurons C, D, E, F, G, H, K, L, M and N, the response

to the leading click (red solid line) remained greater than the response to the lagging click (black solid line) for LLIs of < 2 ms.

Figure 6.1.12 shows the difference in the number of spikes occurring during the leading and lagging click windows during GABA block compared with the control condition for neurons C to O. For 9 of the 13 neurons (E, F, G, I, J, K, M, N, & O; Figure 6.1.12) the increase in the number of spikes occurring during the leading click window (red line) was greater than the increase in the number of spikes occurring during the lagging click window (black line). This was true at all LLIs for all but two neurons (E & G) that showed similar increases in responsiveness to the leading and lagging click at 2 LLIs. The remainder of the neurons (C, D, H, & L) showed approximately equivalent increases in their spike counts in response to the leading and lagging clicks in the GABA block condition compared with the control condition. Therefore, for the majority of neurons described (11/16; includes neurons A, B, & P), responses to the leading click were more suppressed because of GABA-ergic inhibition than responses to the lagging click. For the remaining four neurons (C, D, H, & L), increases in the spike count evoked by the leading and lagging clicks were approximately equivalent during GABA block for most LLIs indicating that there was no difference in the suppressive effect of GABA-ergic inhibition on responses to either the leading or the lagging click for these neurons.

The lagging click recovery functions presented in Figure 6.1.13, Figure 6.1.3 (neuron A), Figure 6.1.6 (neuron B), and Figure 6.1.9 (neuron P) indicate that for neurons A, B, C, E, F, G, H, I, J, N, and O (11/16) that responses to the lagging click did not recover more quickly when GABA was blocked (red lines) compared with the control condition (black lines). For neurons C, E, F, G, H, I, and N there wasn't a consistent effect of blocking GABA on these neurons recovery functions, with recovery at most LLIs approximately equivalent. For neurons A, B, I, J, and O the effect of blocking GABA resulted in less recovery of responsiveness to the lagging click for a number of LLIs (neuron A, 4, 8, & 14-20 ms LLI; neuron B, 4-10 & 18-20 ms LLI; neuron I, 2-14 ms LLI; neuron J, 2-18 ms LLI; neuron O, 2-20 ms LLI), while for the remaining LLIs there was little change in recovery. Blocking GABA resulted in an increase in responsiveness to the lagging click for a number of LLIs in neurons D, K, L, M, and P (5/16). For neurons K and M the increase in responsiveness to the lagging click was

less than 25 % in the GABA block compared with the control condition. For neuron K, recovery of the response to the lagging click was below 30% at all LLIs in both the control and GABA block conditions. For neuron M, maximum recovery of the lagging click response reached 50% at an LLI of 20 ms in the GABA block condition. In the control condition the maximum recovery achieved was 35% at an LLI of 12 ms. Neuron L exhibited robust recovery of its response to lagging click in both the control and GABA block conditions. For and LLI of 20 ms recovery was 95% in the GABA block condition compared with 60% in the control condition. Neuron D also exhibited robust recovery of its response to lagging click in both the control and GABA block conditions. For this neuron the maximum increase in recovery was 45% at an LLI of 12 ms. At an LLI of 20 ms the response of neuron D has recovered to 60% in the GABA block condition compared with 40% in the control condition. For all neurons in which there was an increase in recovery of response the lagging click as a result of blocking GABA there was a response to the lagging click in the control condition (red vs black lines). This indicates that while existing responses to the lagging click increased in the GABA block compared with the control condition for neurons D, K, L, M and P, recovery of responses to the lagging click did not occur at shorter LLIs.

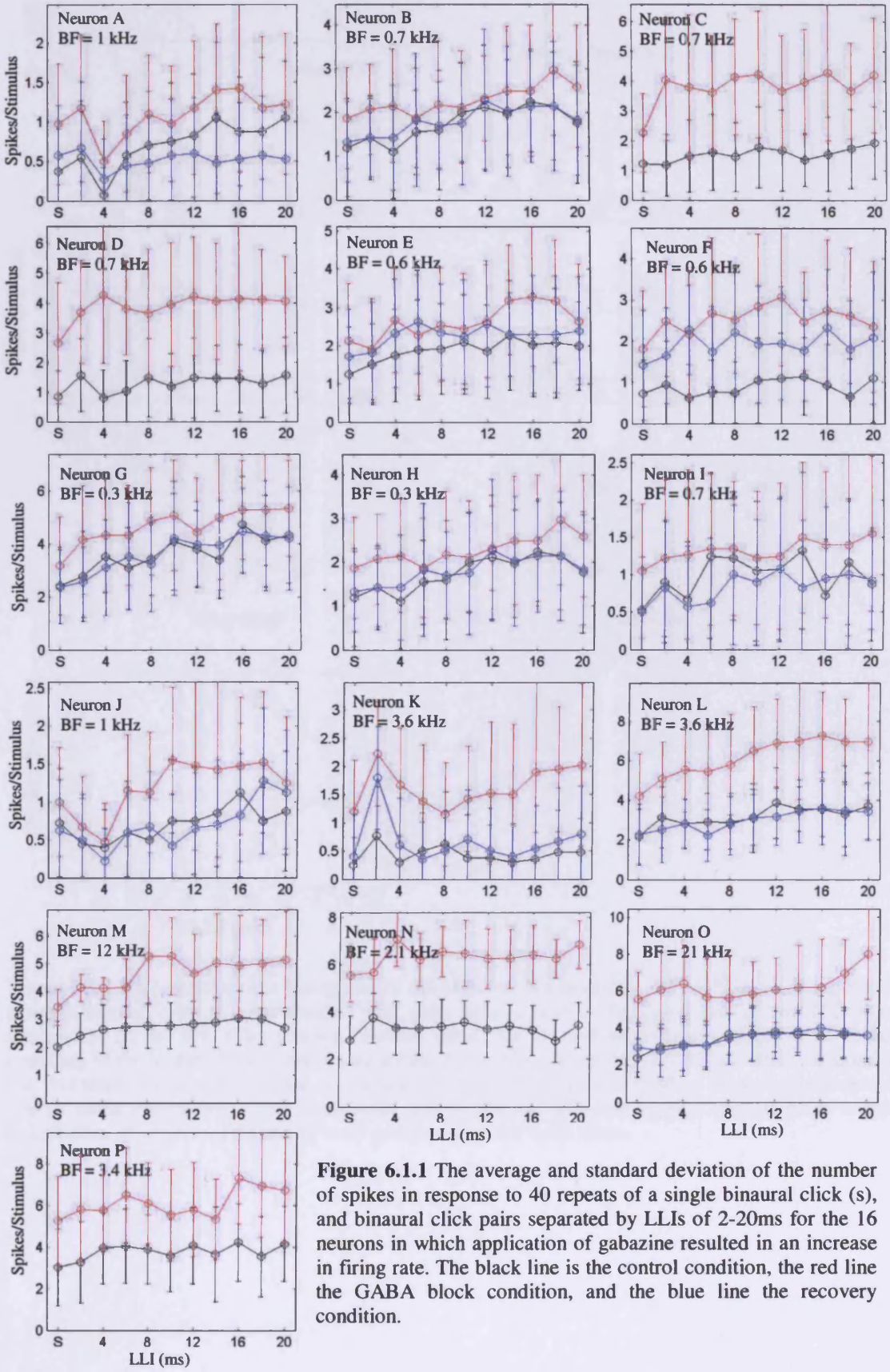


Figure 6.1.1 The average and standard deviation of the number of spikes in response to 40 repeats of a single binaural click (s), and binaural click pairs separated by LLIs of 2-20ms for the 16 neurons in which application of gabazine resulted in an increase in firing rate. The black line is the control condition, the red line the GABA block condition, and the blue line the recovery condition.

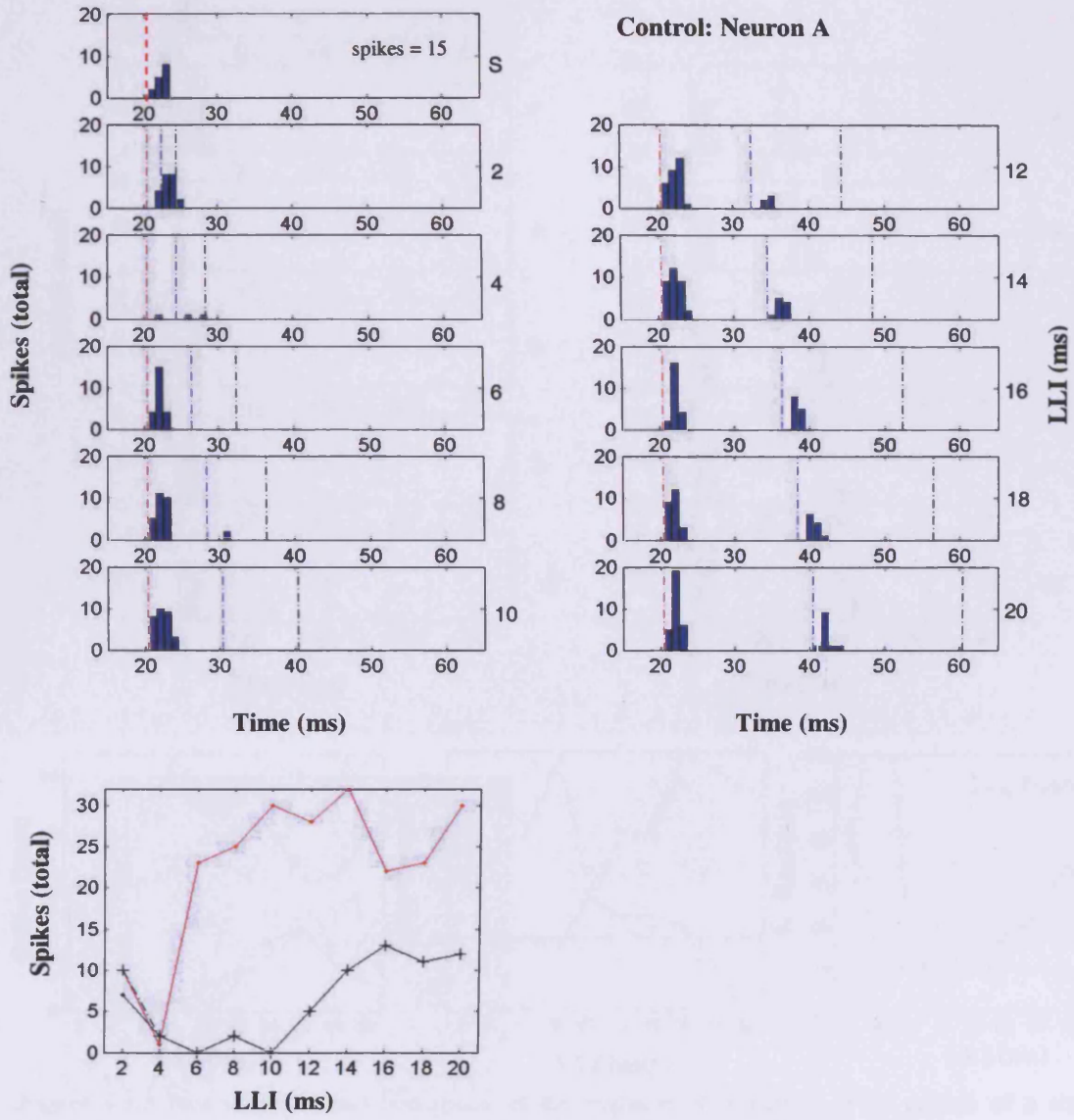


Figure 6.1.2 Post stimulus time histograms of the response of a neuron A (BF = 1kHz) to 40 repeats of a single binaural click (S) and binaural click pairs with LLIs of 2-20ms presented at best ITD at an intensity of 55 dB SPL (Top panels). Bottom panel, the red line represents the number of spikes occurring in the leading click window (red dotted line to blue dotted line on PSTHs). The dotted black line represents the number of spikes occurring during the lagging click window response (blue dotted line to black dotted line). The black solid line shows the adjusted lagging click window count. Calculations of window spike counts were preformed on raw spike times.

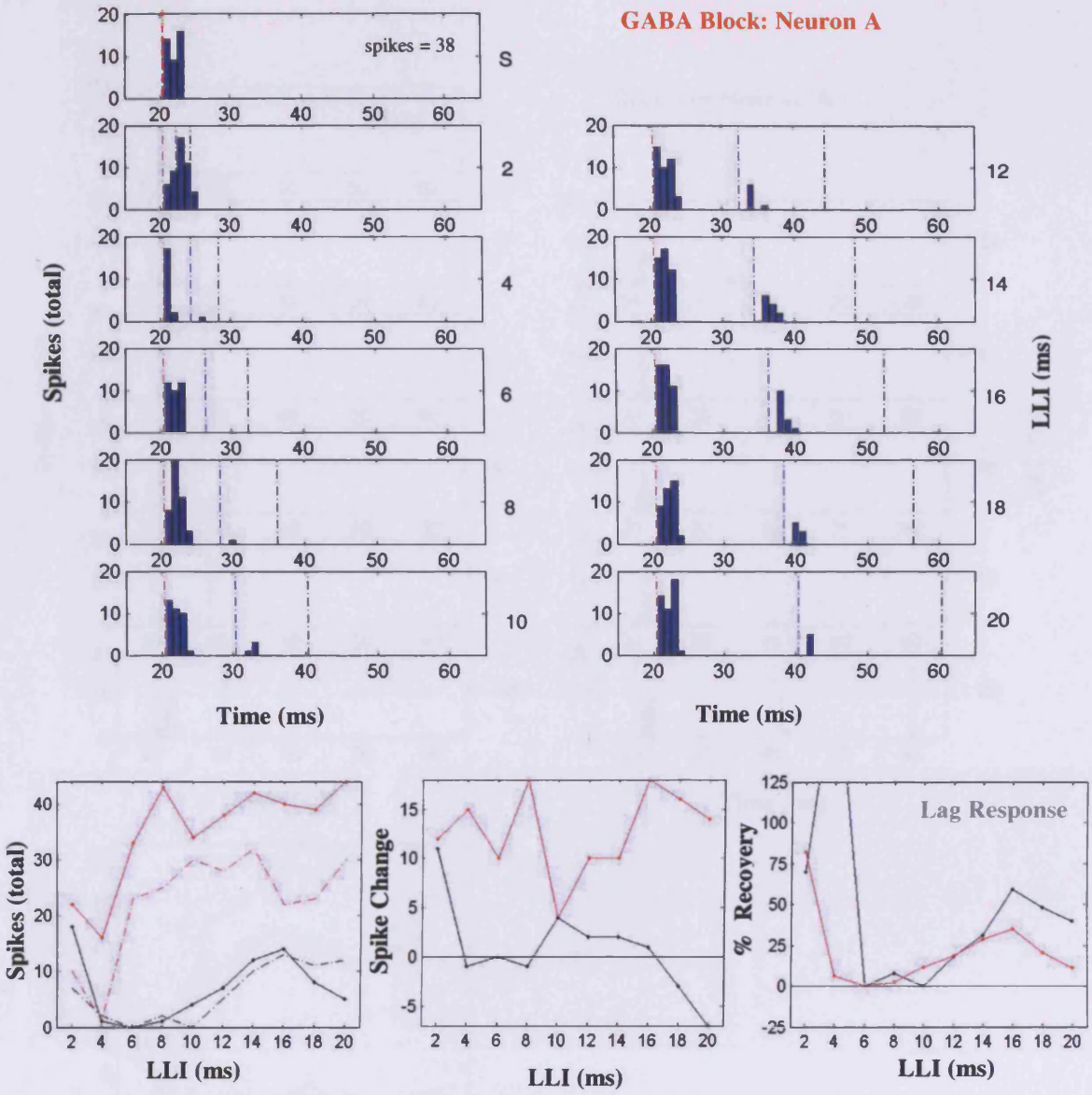


Figure 6.1.3 Post stimulus time histograms of the response of neuron A to 40 repeats of a single binaural click (S) and binaural click pairs with LLIs of 2-20ms presented at best ITD at an intensity of 55 dB SPL (Top panels) under application of gabazine. Bottom left panel, the red line represents the number of spikes occurring during the leading click window (red dotted line to blue dotted line on PSTHs). The solid black line represents the number of spikes occurring during the lagging click window (blue dotted line to black dotted line on PSTHs). The dotted lines show the corresponding values from the control condition. The bottom middle panel shows the difference in spikes evoked by the lead click (red line) and the lag click (black line) in the drug condition compared with the control condition. The bottom right panel shows the lagging click recovery function for the control (black) and GABA block condition (red).

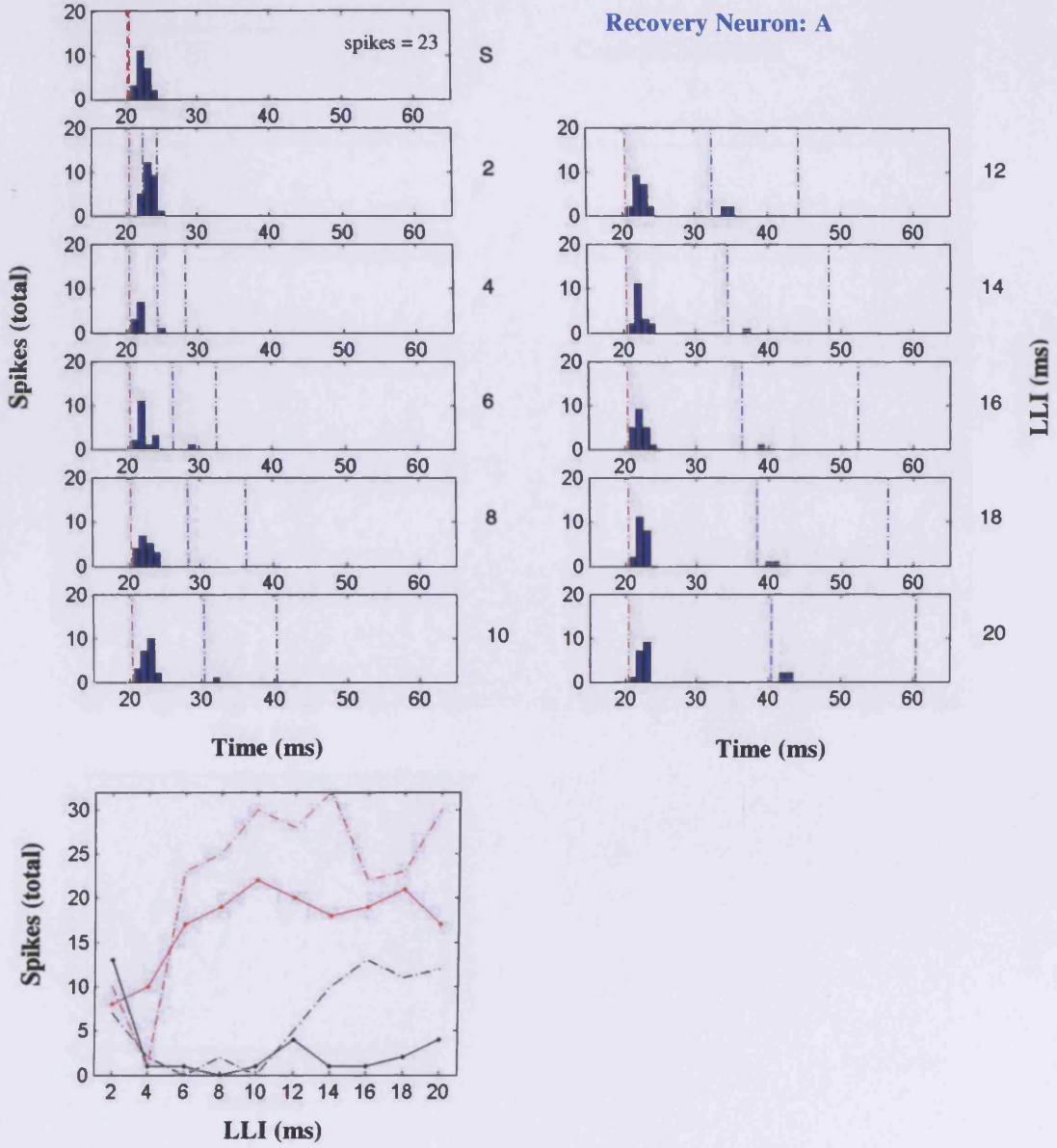


Figure 6.1.4 Post stimulus time histograms of the response of Neuron A to 40 repeats of a single binaural click (S) and binaural click pairs with LLIs of 2-20ms (Top panels), following termination of application of gabazine. Bottom panel, the red solid line represents the number of spikes occurring during the leading click window (red dotted line to blue dotted line on PSTHs). The black solid line represents the adjusted number of spikes occurring during the lagging click window. The dotted lines are the corresponding spike counts from the control condition.

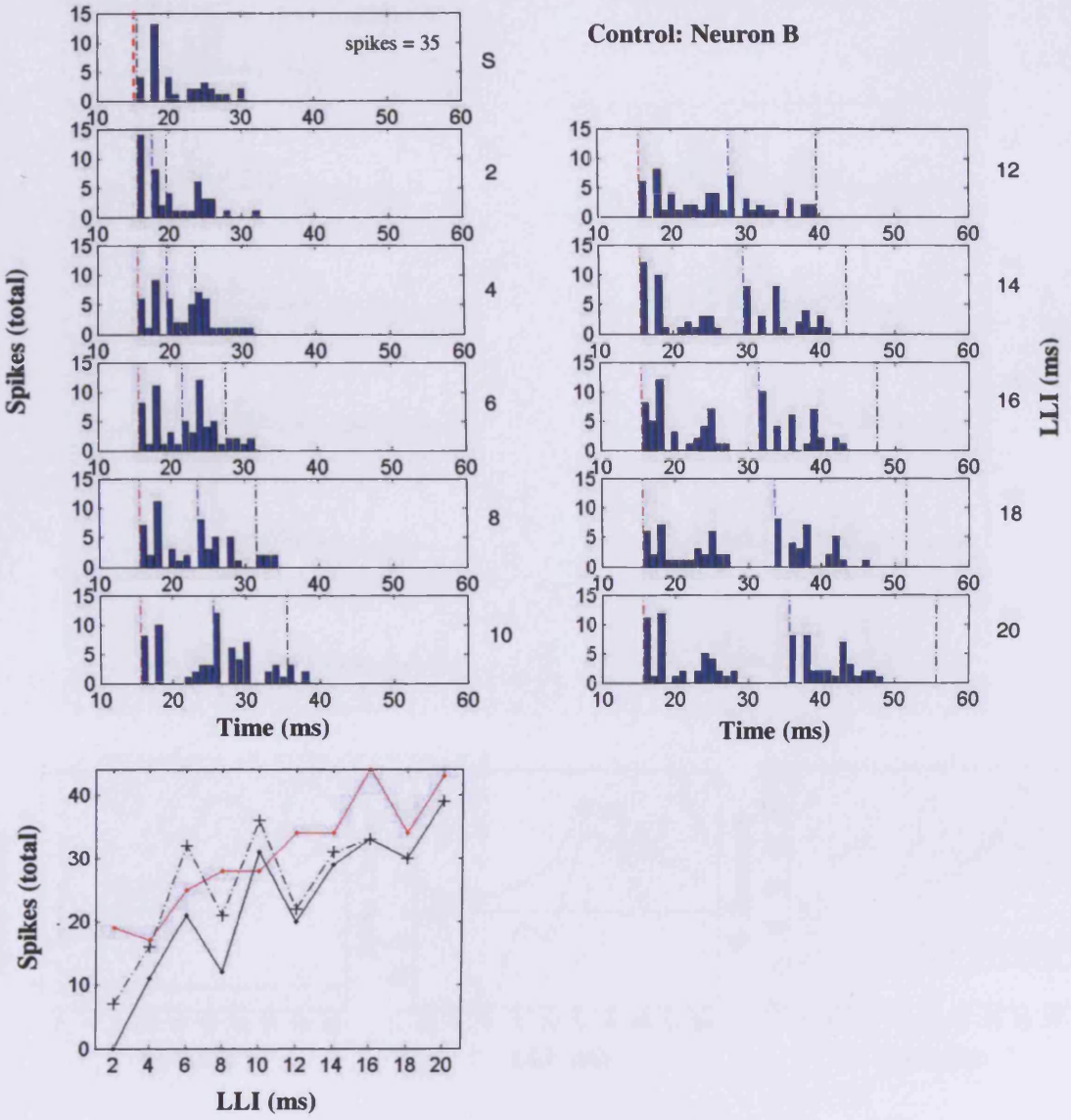


Figure 6.1.5 Post stimulus time histograms of the response neuron B to 40 repeats of a single binaural click (S) and binaural click pairs with LLIs of 2-20ms. Bottom panel, the red line represents the total number of spikes to the lead click summed over window from post stimulus onset to the arrival of the lagging click (red dotted line to blue dotted line on PSTHs). The black line represents the adjusted number of spikes in response to the lagging click, the dotted line the response prior to adjustment. Actual calculations were preformed on raw spike times, not binned PSTH spike times.

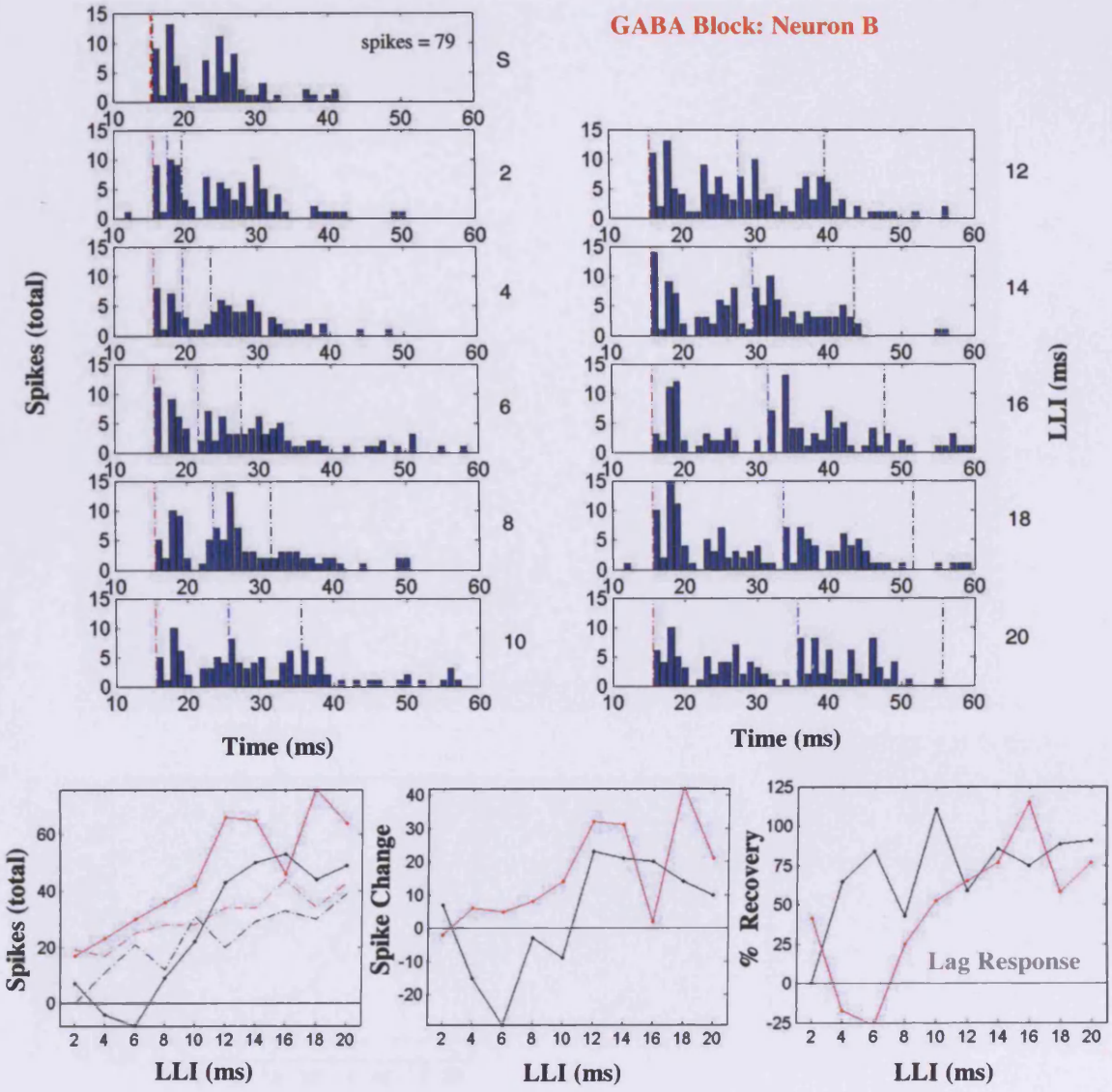


Figure 6.1.6 Post stimulus time histograms of the response of neuron B to 40 repeats of a single binaural click (S) and binaural click pairs with LLIs of 2-20ms presented at best ITD at an intensity of 35 dB SPL (Top panels) under application of the $GABA_A$ antagonist Gabazine. Bottom panel, the red line represents the total number of spikes to the lead click summed over window from post stimulus onset to the arrival of the lagging click (red dotted line to blue dotted line on PSTHs). The black line represents the total number of spikes in response to the lagging click. The bottom middle panel shows the difference in spikes evoked by the lead click (red line) and the lag click (black line) in the drug condition compared with the control condition. The bottom right panel shows the lagging click recovery function for the control (black line) and GABA block conditions (red line).

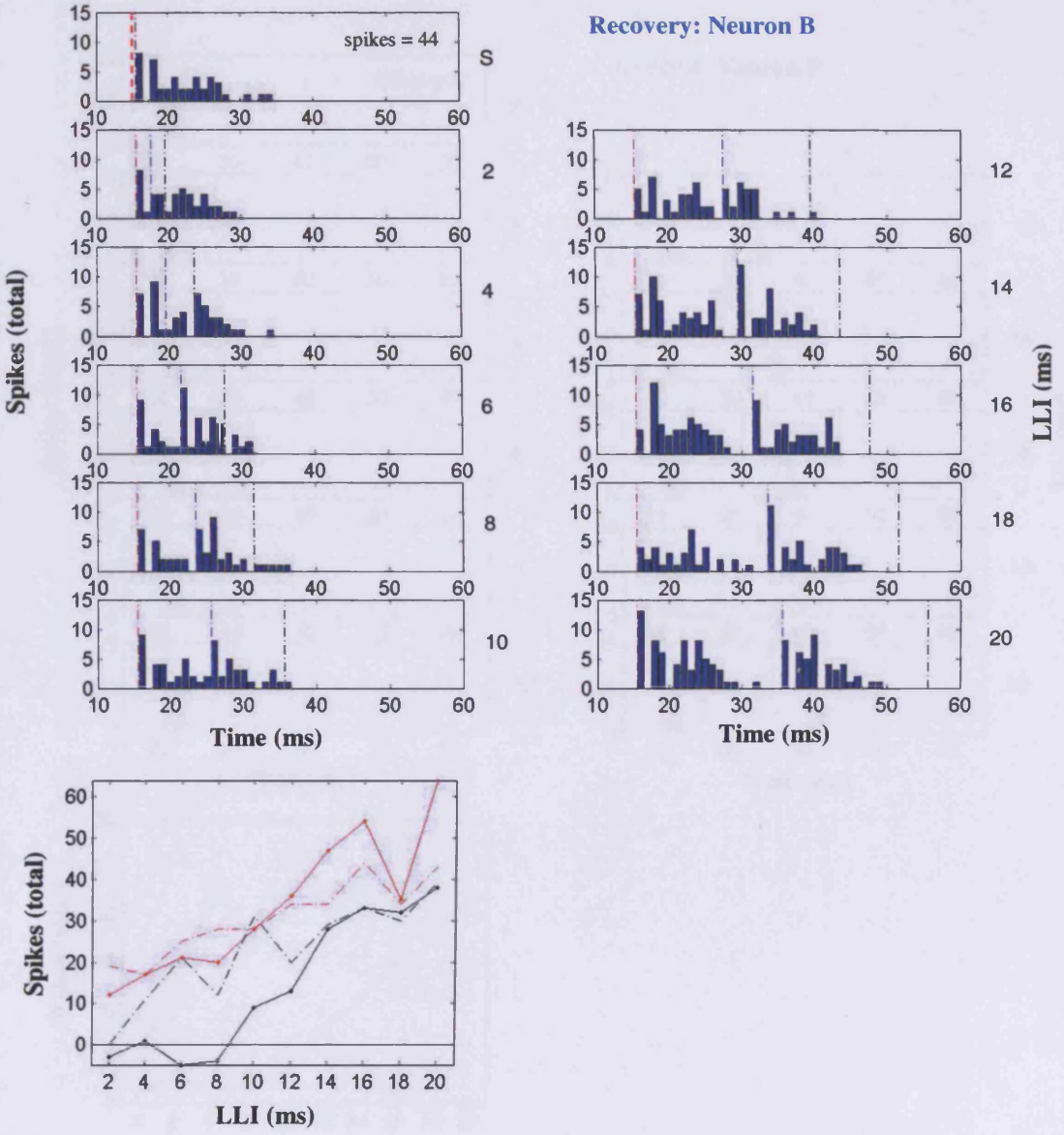


Figure 6.1.7 Post stimulus time histograms of the response of Neuron B to 40 repeats of a single binaural click (S) and binaural click pairs with LLIs of 2-20ms (Top panels), following termination of application of gabazine. Bottom panel, the red solid line represents the number of spikes occurring during the leading click window (red dotted line to blue dotted line on PSTHs). The black solid line represents the adjusted number of spikes occurring during the lagging click window. The dotted lines are the corresponding spike counts from the control condition.

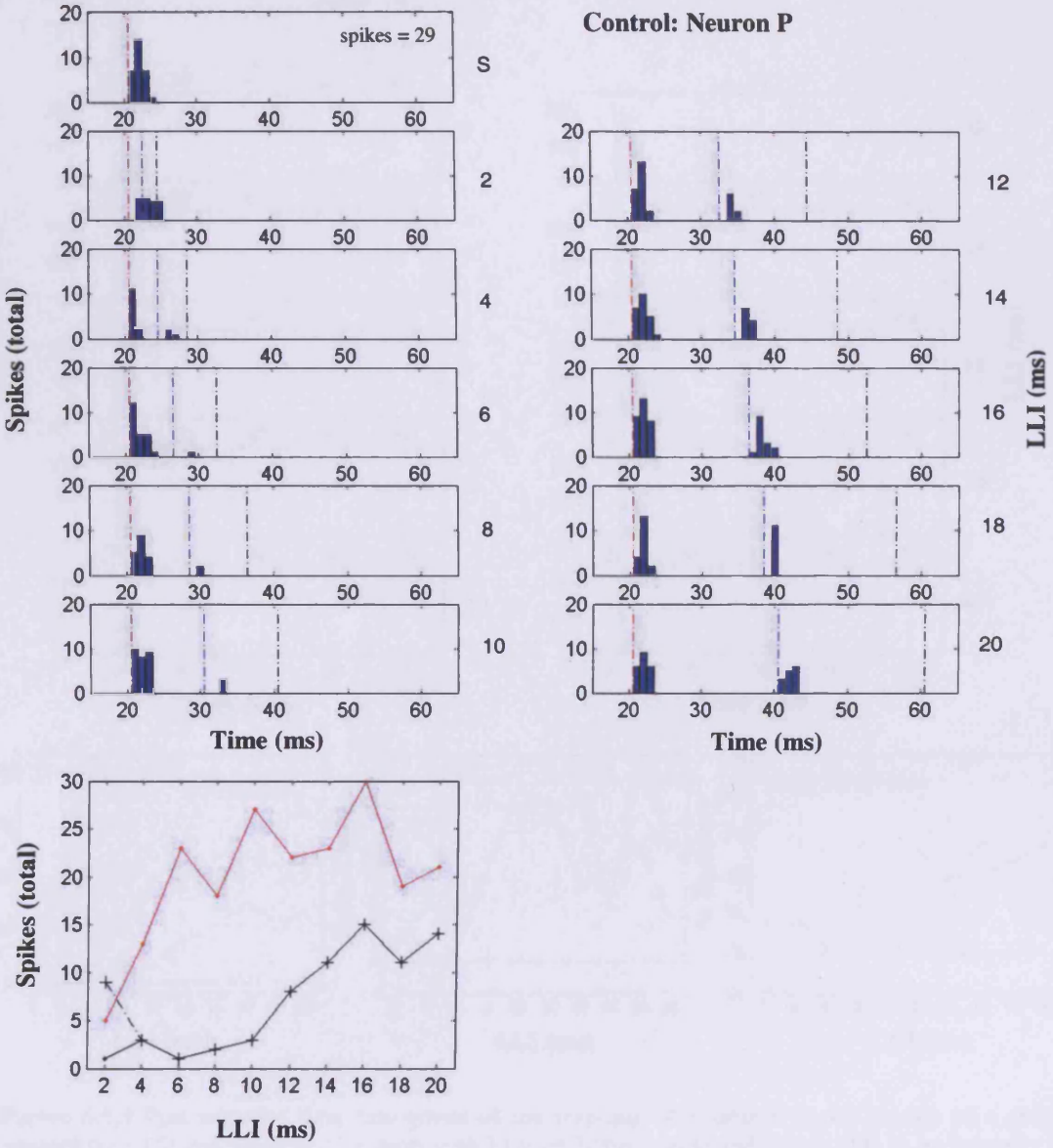


Figure 6.1.8 Post stimulus time histograms of the response neuron B to 40 repeats of a single binaural click (S) and binaural click pairs with LLIs of 2-20ms. Bottom panel, the red line represents the total number of spikes to the lead click summed over window from post stimulus onset to the arrival of the lagging click (red dotted line to blue dotted line on PSTHs). The black line represents the adjusted number of spikes in response to the lagging click, the dotted line the response prior to adjustment. Actual calculations were preformed on raw spike times, not binned PSTH spike times.

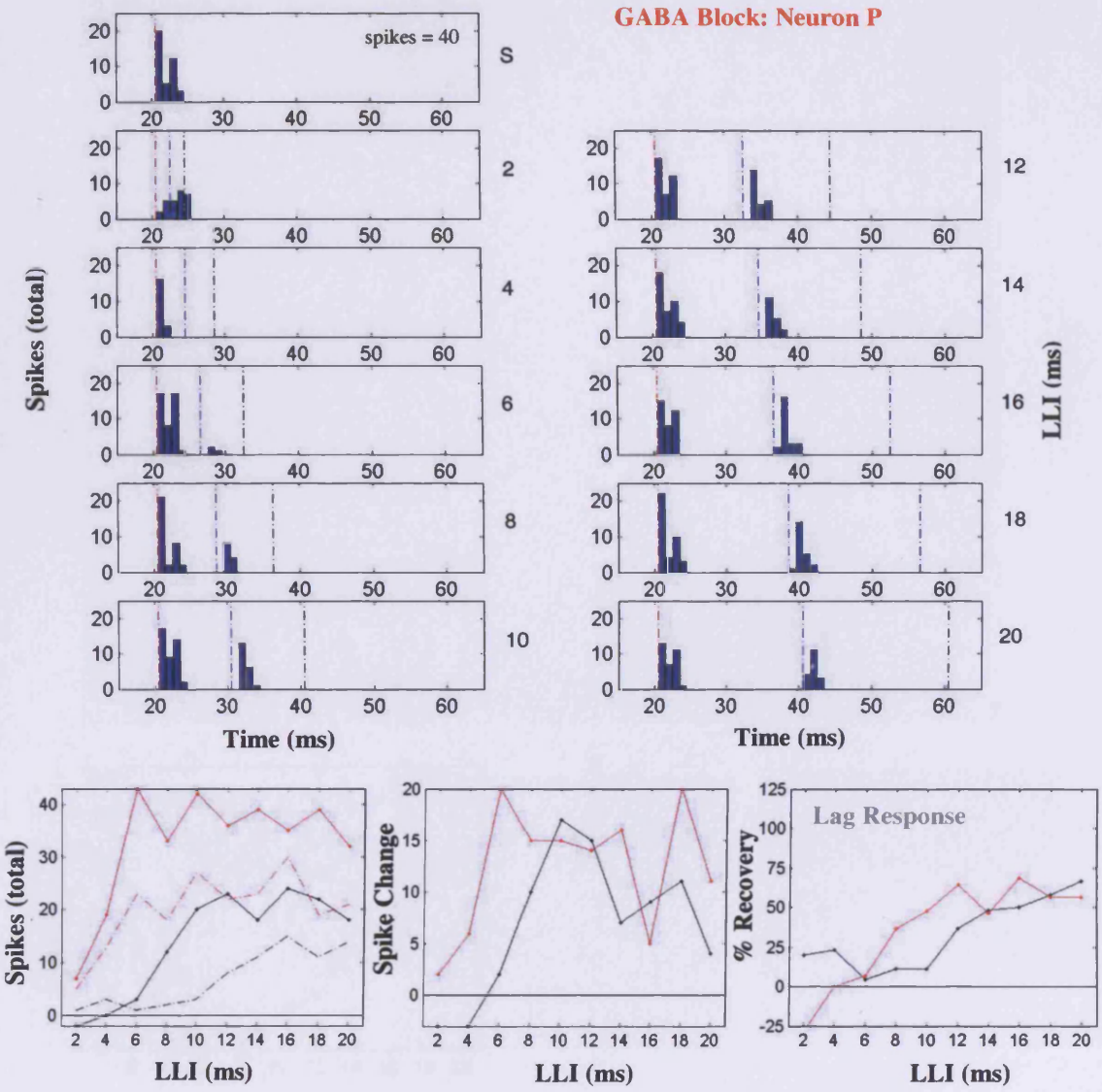


Figure 6.1.9 Post stimulus time histograms of the response of neuron B to 40 repeats of a single binaural click (S) and binaural click pairs with LLIs of 2-20ms presented at best ITD at an intensity of 35 dB SPL (Top panels) under application of the $GABA_A$ antagonist Gabazine. Bottom panel, the red line represents the total number of spikes to the lead click summed over window from post stimulus onset to the arrival of the lagging click (red dotted line to blue dotted line on PSTHs). The black line represents the total number of spikes in response to the lagging click. The bottom middle panel shows the difference in spikes evoked by the lead click (red line) and the lag click (black line) in the drug condition compared with the control condition. The bottom right panel shows the lagging click recovery function for the control (black line) and GABA block conditions (red line).

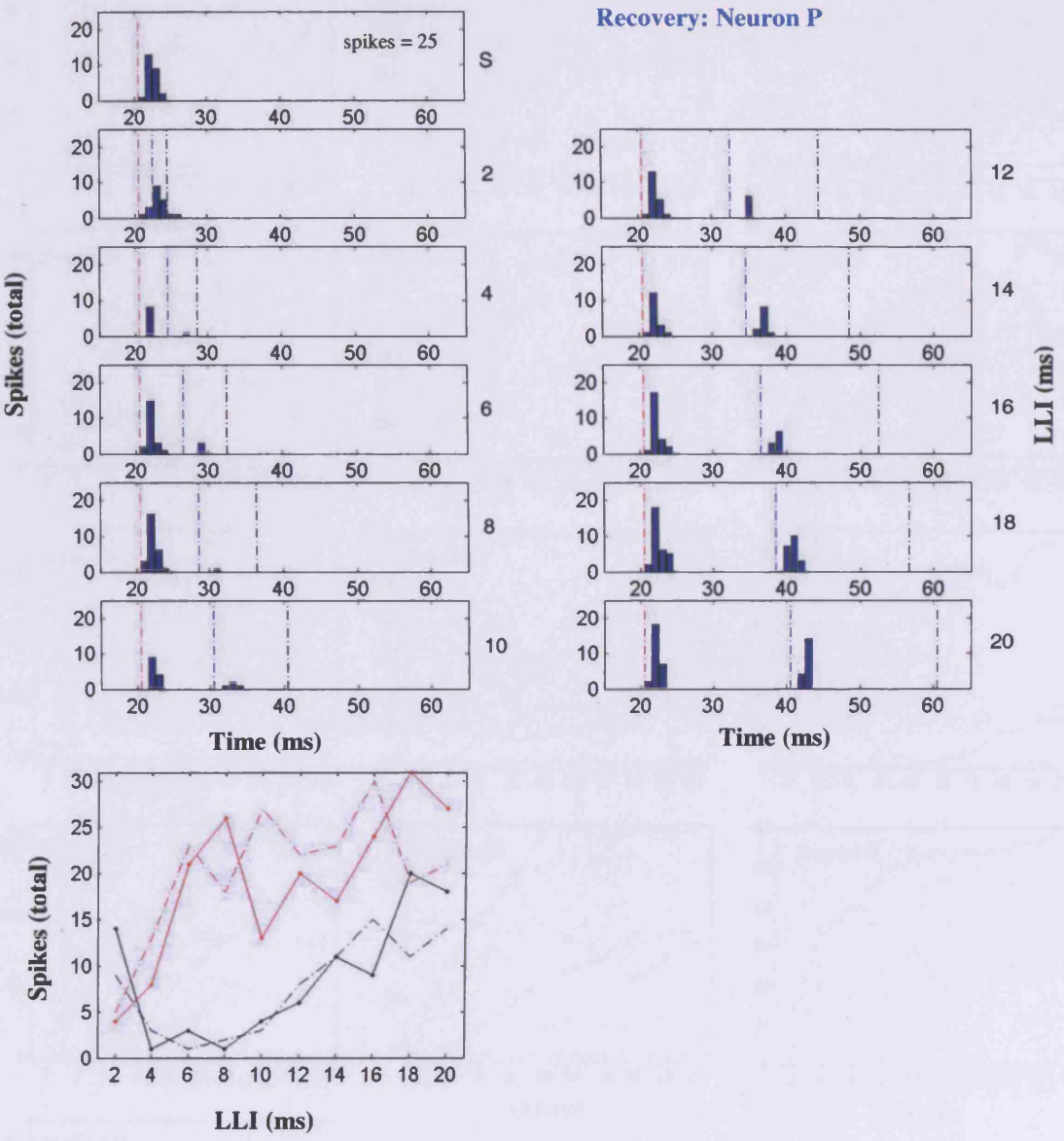


Figure 6.1.10 Post stimulus time histograms of the response of Neuron B to 40 repeats of a single binaural click (S) and binaural click pairs with LLIs of 2-20ms (Top panels), following termination of application of gabazine. Bottom panel, the red solid line represents the number of spikes occurring during the leading click window (red dotted line to blue dotted line on PSTHs). The black solid line represents the adjusted number of spikes occurring during the lagging click window. The dotted lines are the corresponding spike counts from the control condition.

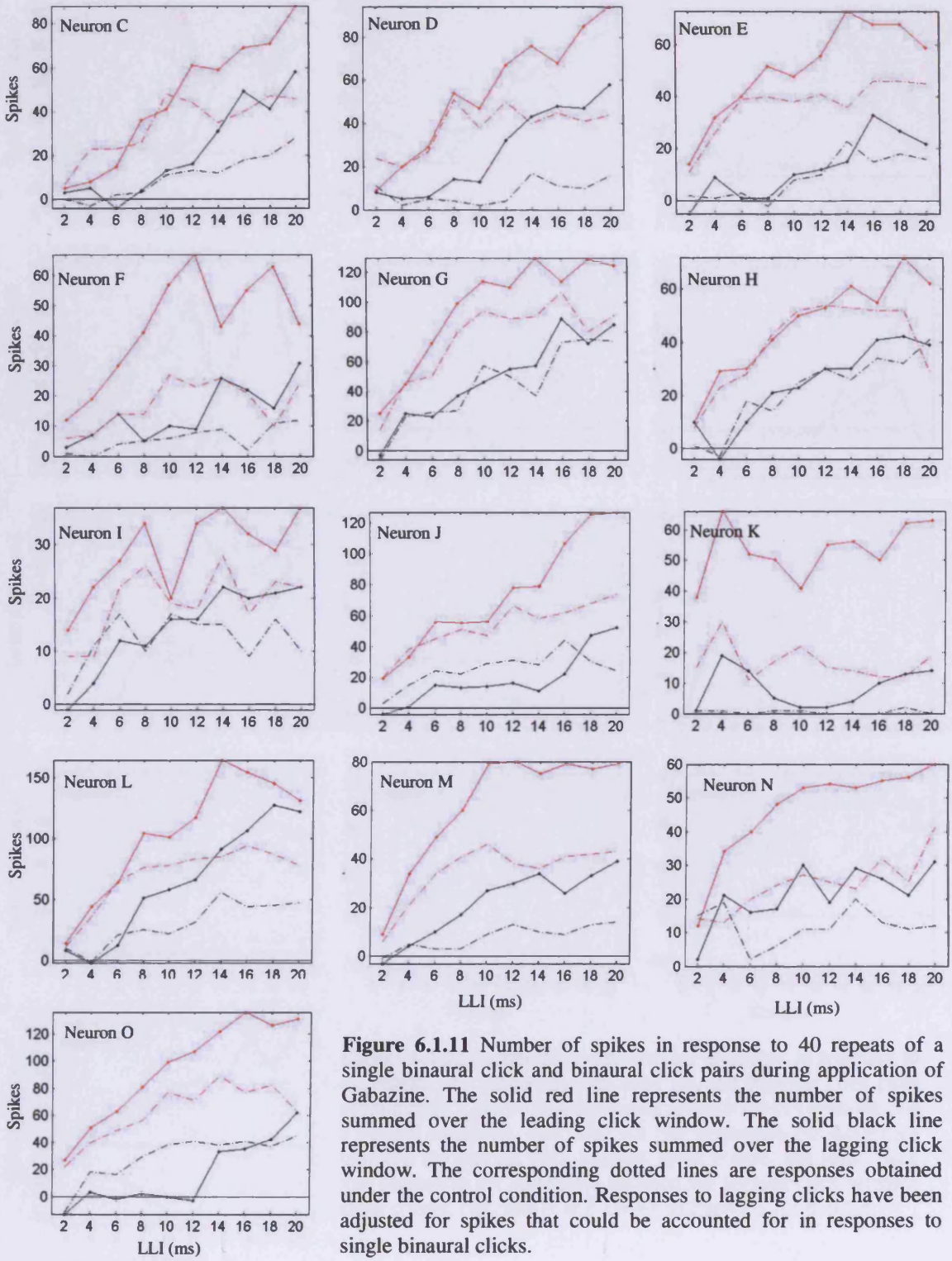


Figure 6.1.11 Number of spikes in response to 40 repeats of a single binaural click and binaural click pairs during application of Gabazine. The solid red line represents the number of spikes summed over the leading click window. The solid black line represents the number of spikes summed over the lagging click window. The corresponding dotted lines are responses obtained under the control condition. Responses to lagging clicks have been adjusted for spikes that could be accounted for in responses to single binaural clicks.

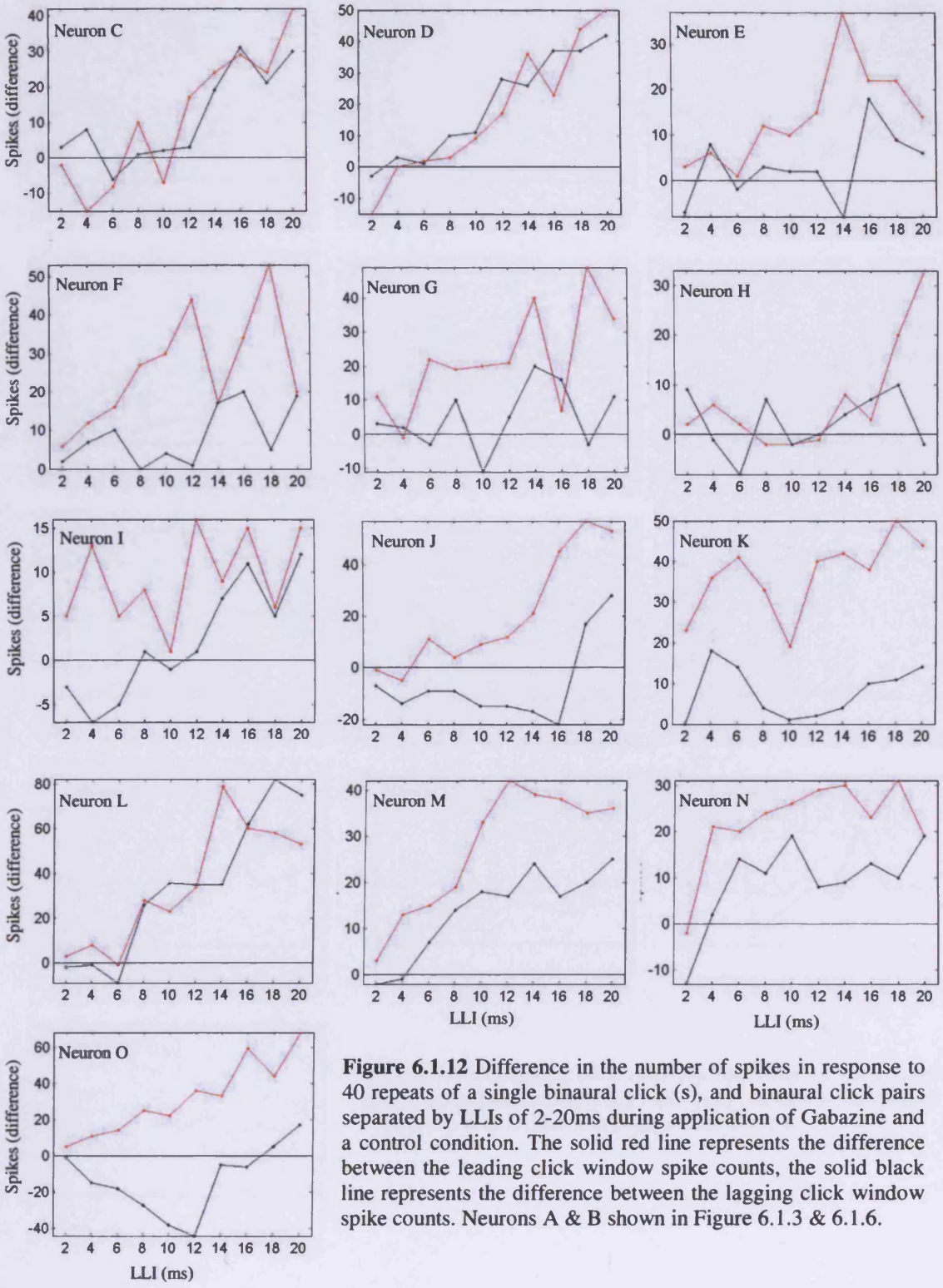


Figure 6.1.12 Difference in the number of spikes in response to 40 repeats of a single binaural click (s), and binaural click pairs separated by LLIs of 2-20ms during application of Gabazine and a control condition. The solid red line represents the difference between the leading click window spike counts, the solid black line represents the difference between the lagging click window spike counts. Neurons A & B shown in Figure 6.1.3 & 6.1.6.

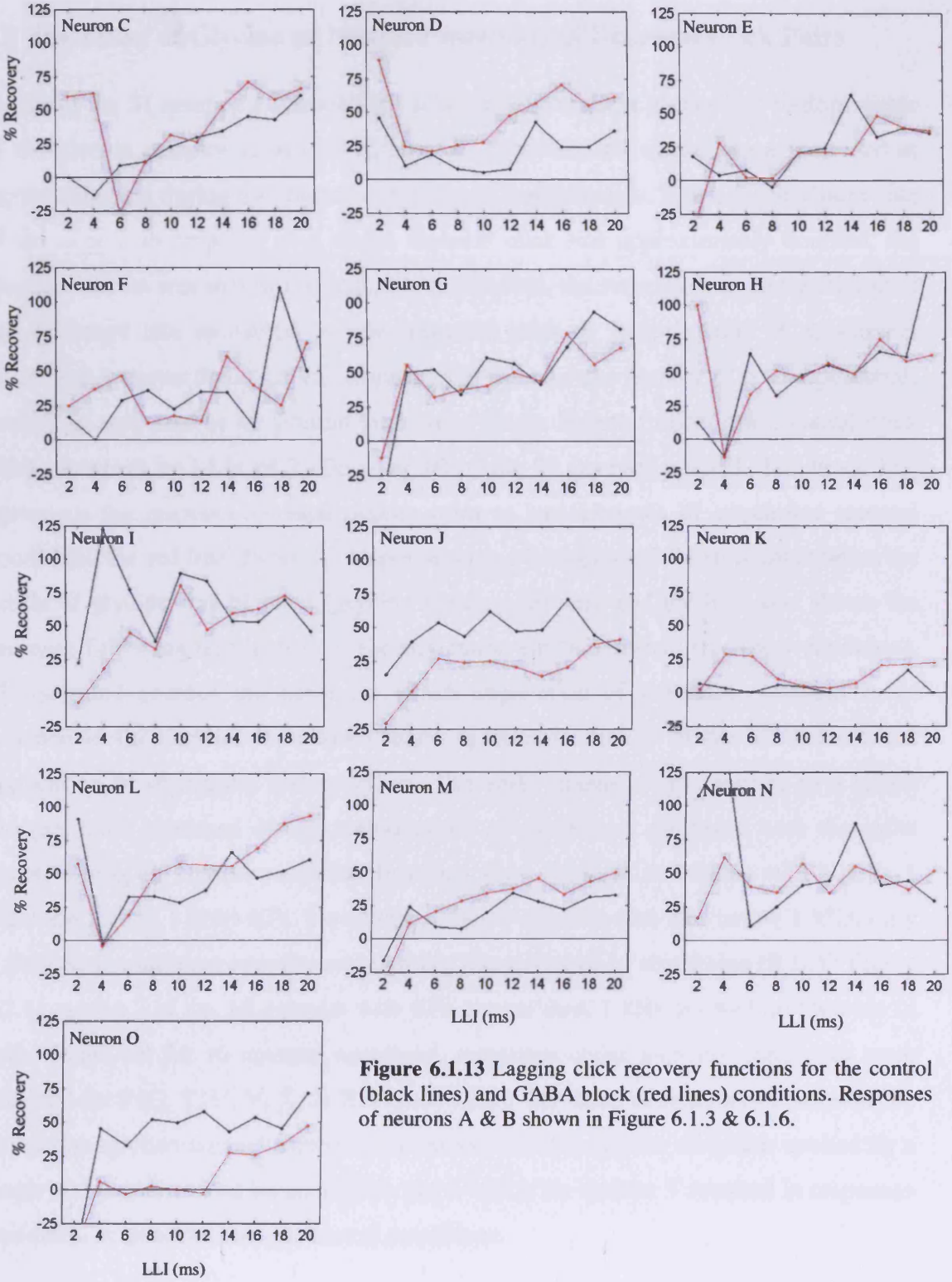


Figure 6.1.13 Lagging click recovery functions for the control (black lines) and GABA block (red lines) conditions. Responses of neurons A & B shown in Figure 6.1.3 & 6.1.6.

6.2 The Effect of Glycine on Neural Processing of Binaural Click Pairs

For 27 of the 51 neurons examined, the action of glycine was blocked by iontophoresis of the glycine receptor antagonist strychnine. Experimental stimuli were presented at regular intervals during the course of strychnine iontophoresis. When the discharge rate of the neuron in response to a single binaural click had approximately doubled, the ejection current was terminated and, where possible, the response was monitored until the discharge rate recovered to rate observed prior to iontophoresis of strychnine. Figure 6.2.1 shows the mean and standard deviation of the number of action potentials evoked in response to 40 presentations of a single binaural click and binaural click pairs separated by LLIs of 2-20ms for 10 of the 27 neurons studied. The black line represents the response of each neuron prior to iontophoresis of strychnine (control condition), the red line shows the response during iontophoresis of strychnine when the action of glycine was blocked (glycine block condition), and the blue line shows the response following termination of the strychnine ejection current (recovery condition). The selected neurons are those for which application of strychnine resulted in an increase in the number of spikes evoked by stimuli. For 17 of the 27 neurons the application of strychnine had no effect. The spike counts of 10 neurons to a single binaural click increased during iontophoresis of strychnine, compared with the spike counts during the control condition. Increases were observed in neurons with high (> 1 kHz) and low (< 1 kHz) BFs. However, of the 11 neurons with BFs below 1 kHz, only 3 showed any increase in spike count during iontophoresis of strychnine (R,S,Y; Figure 6.2.1), whilst 7 of the 16 neurons with BFs greater than 1 kHz showed an increase in spike count. Of the 10 neurons examined, responses under recovery conditions were obtained for 7 (Q, T, U, V, X, & Z; Figure 6.2.1). For these neurons termination of the strychnine ejection current resulted in a reduction in the number of spikes evoked by a single binaural click and binaural click pairs, which for neuron V resulted in responses equivalent to those seen under control conditions.

6.2.1 Neuron W

Neuron W provides an example of a neuron for which glycinergic inhibition suppressed the neurons response to the lagging click of a binaural click pair by an equivalent or greater amount than its response to the leading click. The top panels of Figure 6.2.2 show the responses of neuron W (BF = 8.6 kHz) as a series of post stimulus time histograms (PSTHs) of the number of spikes evoked in response to a single binaural click and binaural click pairs separated by LLIs of 2-20 ms. The red dotted line on the PSTHs indicate the start of the leading click response window and is the neurons response latency when presented with a single binaural click. For the panels showing the response to binaural click pairs, the blue dotted line indicates the arrival of the lagging click relative to the neurons response latency and marks the end of the leading click response window and the start of the lagging click response window. The black dotted line indicates the equivalent period between onset and the arrival of the lagging click and marks the end of the lagging click response window. When presented with a single binaural click, neuron W showed an increase in the number of spikes above its spontaneous activity that had a central peak in the spike distribution. Following the response to a single binaural click or binaural click pair, the spontaneous activity was suppressed for a period of approximately 10ms after which it reappeared at approximately 30 ms. For binaural click pairs separated by LLIs of 2-10ms there was little change in the spike distribution of the response of neuron W from that to a single binaural click although the period for which the spontaneous activity was suppressed subsequent to the response to a single binaural click lengthened for LLIs of 8 and 10 ms. At a LLI of 12 ms a response coinciding with the arrival of the lagging click emerged and increased in magnitude with increasing LLI.

The panel in the bottom left of Figure 6.2.2 shows the number of spikes that occurred during the leading click window (red line) and the number of spikes that occurred during the lagging click window following adjustment for the response to the leading click. Comparison of the red and black lines shows the neurons response to the leading click was greater than its response to the lagging click for all LLIs. The neurons response to the lagging click increased for LLIs in the range 12-18 ms, approaching the spike count during the leading click window for LLIs of 18 and 20 ms.

Figure 6.2.3 shows the response of neuron W to a single binaural click and to binaural click pairs during iontophoresis of strychnine. Iontophoresis of strychnine resulted in an increase in the neurons response to a single binaural click from 24 spikes to 32 spikes (top left panel, Figure 6.2.3), indicating the efficacy of the intervention. In response to binaural click pairs the PSTHs in the top panels of Figure 6.2.3 show the neuron responded robustly to the leading click at all LLIs, and showed a suppressed response to the lagging click for LLIs of 8-12 ms. This contrasts with the neurons response in the control condition (Figure 6.2.2) in which a response to the lagging click did not emerge until a LLI of 12 ms. By an LLI of 14 ms the response appeared equivalent to the neurons response to the leading click (Figure 6.2.3).

The bottom left panel of Figure 6.2.3 shows the number of spikes occurring during the leading (red solid line) and lagging (black solid line) click windows for LLIs of 2-20 ms. The responses obtained for the leading and lagging click windows in the control condition are represented by the corresponding dotted lines. The response of this neuron to both the leading and lagging click was greater in the glycine block condition than in the control condition for LLIs of > 2 ms. The bottom middle panel shows the difference in the spike count derived from the leading click window (red line) and lagging click response window in the glycine block condition compared with the control condition. For this neuron, the response to the lagging click increased by a greater amount than to the leading click for LLIs of 10-16 ms and was approximately equivalent for all other LLIs > 2ms. This indicates that glycine suppressed the response to the lagging click by an equivalent or greater amount than the response to the lagging click.

The bottom right panel of Figure 6.2.3 shows the lagging click recovery functions for neuron W under control (black line) and glycine block (red line) conditions. The recovery functions indicate that for this neuron the leading click suppressed the neurons response to a greater extent in the control condition compared with the glycine block condition. For an LLI of 12 ms, recovery of the lagging click response reached 70% in the glycine block condition and continued to increase for subsequent LLIs. In the control condition, the response to the lagging click was less than 20% of the neurons response to the leading click and did not approach the recovery seen in the glycine block condition until an LLI of 18 ms. The recovery functions show that the greatest

release from suppression occurred for LLIs of 10-16 ms (red vs. black line) indicating that glycinergic inhibition was strongest for these LLIs.

Figure 6.2.4 shows the response of neuron W to a single binaural click and binaural click pairs separated by LLIs of 2-20ms following termination of the strychnine ejection current. The response of the neuron to a single binaural click was 21 spikes which was 3 spikes less than the response in the control condition indicating the effect of the strychnine intervention had reduced. The PSTHs indicate that the response to the lagging click that was present in the glycine block condition from a LLI of 8 ms was not present in the recovery condition until an LLI of 14 ms. The spikes counts derived from the leading and lagging response windows plotted as a function of the LLI in the bottom left panel show the response to the leading click was equivalent to, or less than that obtained in the control condition. The response to the lagging click was slightly less (2-8 & 18-20 ms LLI) than or greater than (10-16 ms LLI) that obtained in the control condition.

6.1.2 Neuron Q

Figure 6.2.5 shows the response of neuron Q to a single binaural click and binaural click pairs separated by LLIs of 2-20 ms. For this neuron glycinergic inhibition suppressed the neurons response to the leading and lagging clicks by approximately the same amount. The PSTHs in the top panels of Figure 6.2.5 show neuron Q responded to a single binaural click with a single period of tightly timed spike activity over the 40 presentations of the stimulus. At an LLI of 8 ms neuron Q exhibited a suppressed responded to the lagging click of the binaural click pair that was present for all subsequent LLIs. The spike counts calculated from the leading and lagging click response windows, plotted in the bottom left panel of Figure 6.2.5, show the response of neuron Q to the leading click (red line) was stable as a function of LLI with the leading click evoking a single spike per presentation of the stimulus. The neurons response to the lagging click, which emerged at an LLI of 8 ms, increased rapidly from 8 to 29 spikes at 10 ms LLI after which the response increased very little.

Figure 6.2.6 shows the response of neuron Q to a single binaural click and binaural click pairs in the glycine block condition. With the action of glycine blocked neuron Q responded more robustly to a single binaural click (60 spikes vs. 39 spikes) and the

leading click of a binaural click pair compared with responses in the control condition, as well as responding more robustly to the lagging click of a binaural click pair. The response to the lagging click emerged at a LLI of 6 ms in the GABA block condition compared with 8 ms in the control condition. The bottom left panel shows the number of spikes occurring during the leading (red solid line) and lagging (black solid line) click windows for LLIs of 2-20ms. The responses obtained for the leading and lagging click windows in the control condition are represented by the corresponding dotted lines. The response of neuron Q during the leading and lagging click window increased at all LLIs in the drug condition compared with the control conditions. However, the response during the lagging click window was always less than that during the leading click window. The bottom middle panel of Figure 6.2.6 shows the difference in the number of spikes evoked by the leading click (red line) and the lagging click (black line) in the glycine block condition compared with the control conditions. The increase in the number of spikes associated with the lead click in the glycine block condition was greater than that during the lagging click window at all LLIs with the exceptions of 8ms and 14ms. Therefore, the glycinergic inhibition arriving with the response during the leading click window was generally greater than that arriving during the lagging click window. This indicates glycine suppressed the neurons response to the leading click more than its response to the lagging click. The lagging click recovery functions presented in the bottom right panel of Figure 6.2.6 for the control (black line) and glycine block conditions (red line) indicate that the leading click suppressed the neurons response to the lagging click to a greater extent in the control condition compared with the glycine block condition.

Figure 6.2.7 shows the responses of neuron Q after termination of the strychnine current. Spike counts in response to both the leading and lagging click were reduced relative to the glycine block condition. The spike count in response to a single binaural click was 41, close to the control value of 39. The bottom left panel showing the spike count to the leading (solid red line) and lagging (solid black line) clicks indicates that, in contrast to the response to a single binaural click, spike counts for the leading and lagging click windows had returned to pre-drug (dotted lines) intensities for short LLIs less than 8 ms but not for longer LLIs. Rather, at long LLIs the responses to the leading and lagging clicks were still increased relative to the control condition.

6.1.3 Group Results

Figure 6.2.8 shows the spike counts obtained to the leading and lagging click windows in response to binaural click pairs separated by LLIs of 2-20ms for the 10 neurons for which there was an increase in responsiveness following application of strychnine. The red lines indicate spike counts during the leading click window under drug (solid line) and control (dotted line) conditions. The black lines indicate spike counts derived from the lagging window under drug (solid line) and control (dotted line) conditions. Under control conditions, the number of spikes occurring during the leading click window was greater than the number occurring during the lagging window for binaural click pairs at all LLIs for all neurons, with the exception of neuron R. Instead, neuron R showed an equivalent response during the leading and lagging click windows for LLIs of 8ms or longer. During glycine block (solid lines) the number of spikes occurring during the leading click window and/or the lagging click window increased relative to the control values for all neurons except neuron X which showed a decrease in both windows. The increase in firing rate observed in the summed response to the leading and lagging click for neuron X (Figure 6.2.1) was due to an increase in spontaneous activity in the drug condition. The increase in spike count due to release from glycinergic inhibition did not produce equivalent responses to the leading and lagging click of a binaural click pair in any neuron.

Figure 6.2.9 shows the difference in the number of spikes occurring during the leading (red line) and lagging (black line) click windows during glycine block compared with the control condition for the sample of neurons. Neurons Q, S, U, V, Y, and Z all show either a greater increase in their spike count in response to the leading click than the lagging click or approximately equivalent increases to both. As described above, neuron X showed a reduced spike count during the leading and lagging click windows during glycine block. In contrast, neurons T, W and R all showed greater increases during the lagging click window compared with the leading click window for several consecutive LLIs. This pattern of increases resulted in a more robust representation of the lagging click at earlier LLIs than was the case in the control condition (Exemplified by Neuron W; Figure 6.2.2-6.2.4).

Figure 6.2.10 shows the lagging click recovery function for the control (black lines) and glycine block conditions (red lines). Comparison of the recovery functions from each condition indicates that for 8 out of 10 of the neurons presented blocking glycine did not decrease the suppression caused by the leading click. For neurons T, V and W (example neuron) blocking the action of glycine did decrease the suppression caused by the lagging click. For both neurons the decrease in suppression was greatest for LLIs > than 12 ms.

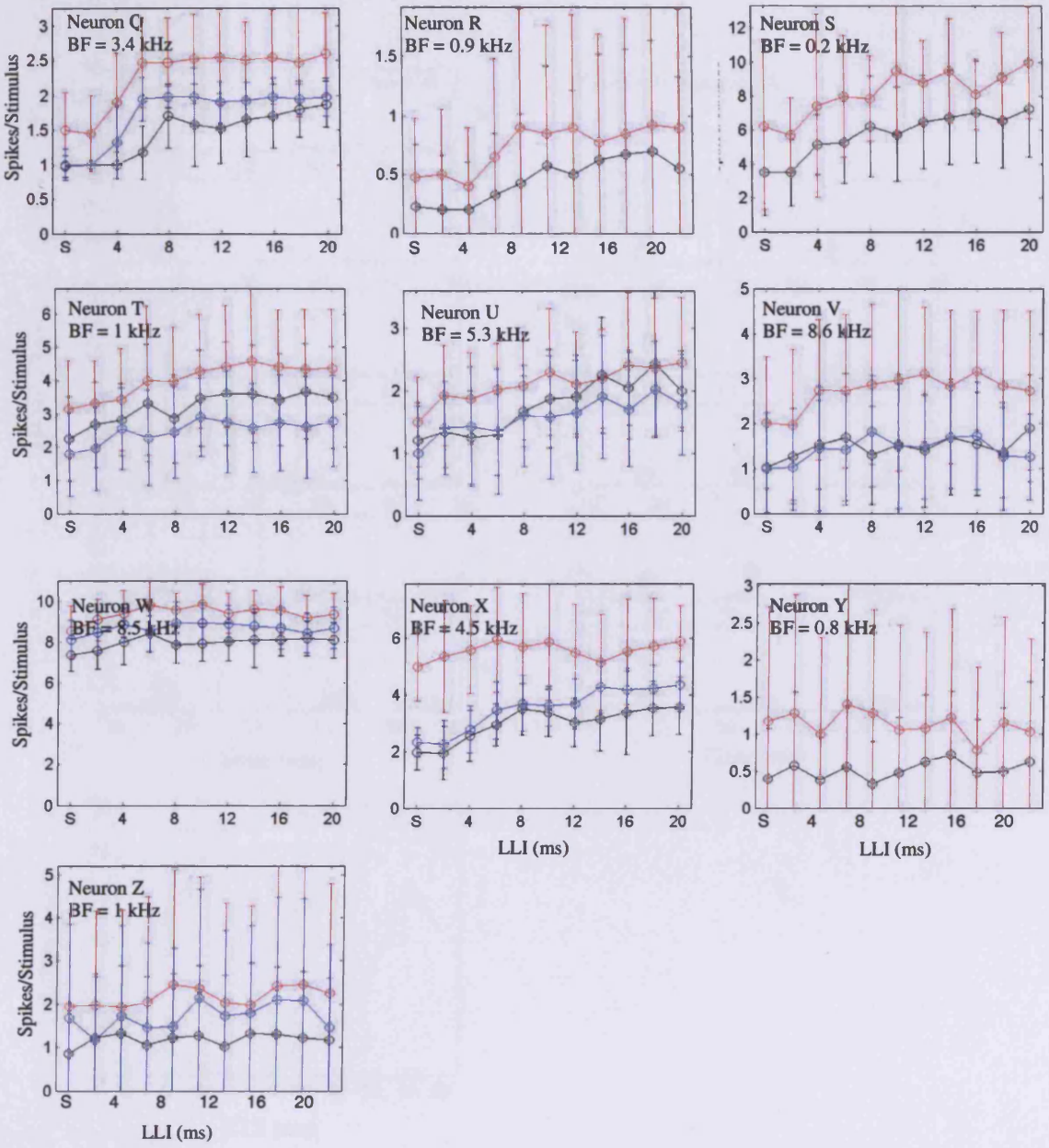


Figure 6.2.1 The average and standard deviation of the number of spikes in response to 40 repeats of a single binaural click (s), and binaural click pairs separated by LLIs of 2-20ms for the 10 neurons in which application of strychnine resulted in an increase in firing rate. The black line is the control condition, the red line the glycine block condition, and the blue line the recovery condition.

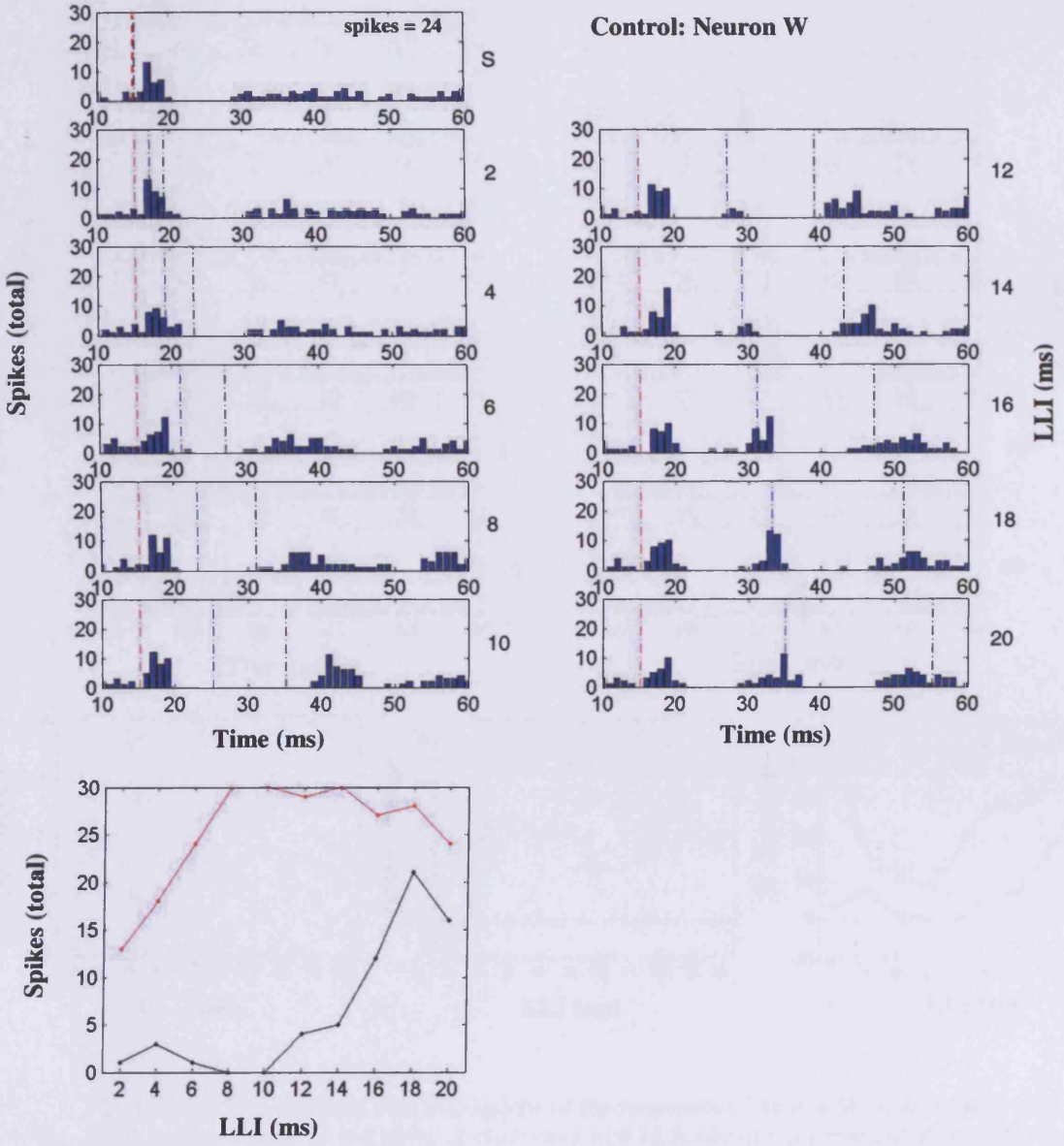


Figure 6.2.2 Post stimulus time histograms of the response of neuron W to 40 repeats of a single binaural click (S) and binaural click pairs with LLIs of 2-20ms. Bottom panel, the red line represents the total number of spikes to the lead click summed over window from post stimulus onset to the arrival of the lagging click (red dotted line to blue dotted line on PSTHs). The black line represents the adjusted number of spikes in response to the lagging click. Actual calculations were preformed on raw spike times, not binned PSTH spike times.

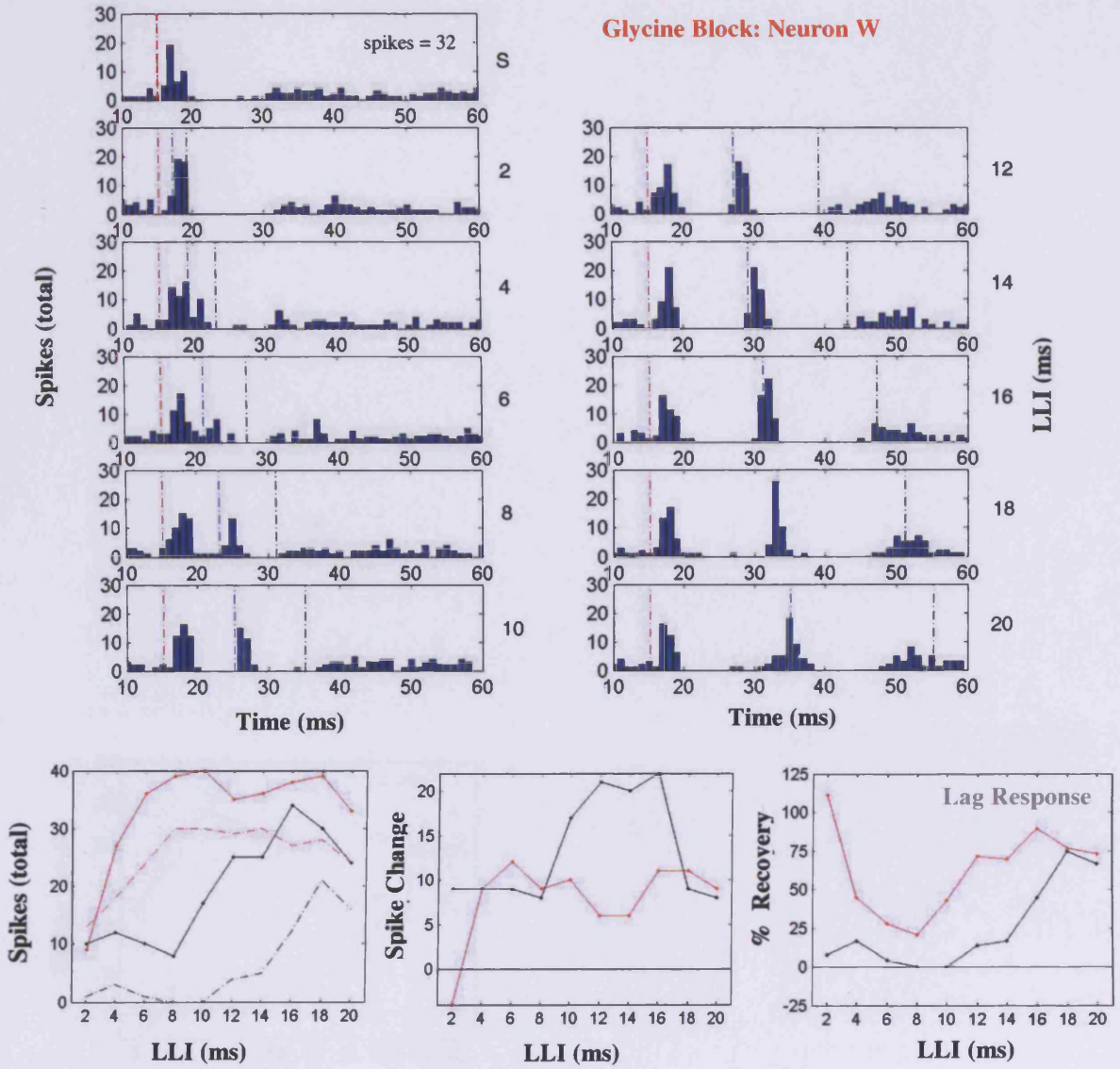


Figure 6.2.3 Post stimulus time histograms of the responses of neuron W to 40 repeats of a single binaural click (S) and binaural click pairs with LLIs of 2-20ms presented at best ITD at an intensity of 35 dB SPL (Top panels) under application strychnine. Bottom panel, the red line represents the total number of spikes to the lead click summed over window from post stimulus onset to the arrival of the lagging click (red dotted line to blue dotted line on PSTHs). The black line represents the total number of spikes in response to the lagging click. The bottom middle panel shows the difference in spikes evoked by the lead click (red line) and the lag click (black line) in the GABA Block condition compared with the control condition. The bottom right panel shows the lagging click recovery functions for the control (black line) and GABA block conditions (red line).

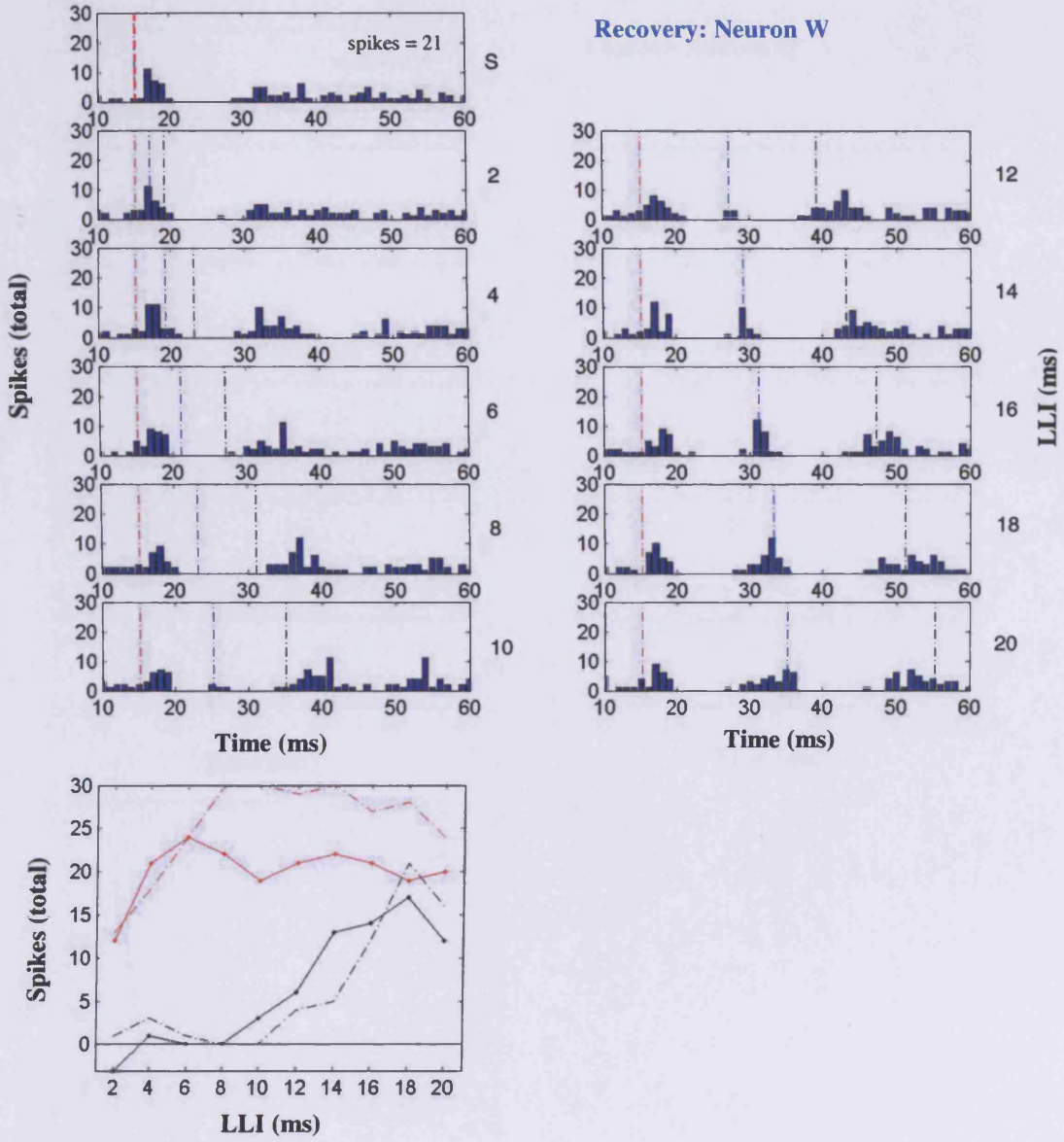


Figure 6.2.4 Post stimulus time histograms of the response of Neuron W to 40 repeats of a single binaural click (S) and binaural click pairs with LLIs of 2-20ms (Top panels), following termination of application of strychnine. Bottom panel, the red solid line represents the number of spikes occurring during the leading click window (red dotted line to blue dotted line on PSTHs). The black solid line represents the adjusted number of spikes occurring during the lagging click window. The dotted lines are the corresponding spike counts from the control condition.

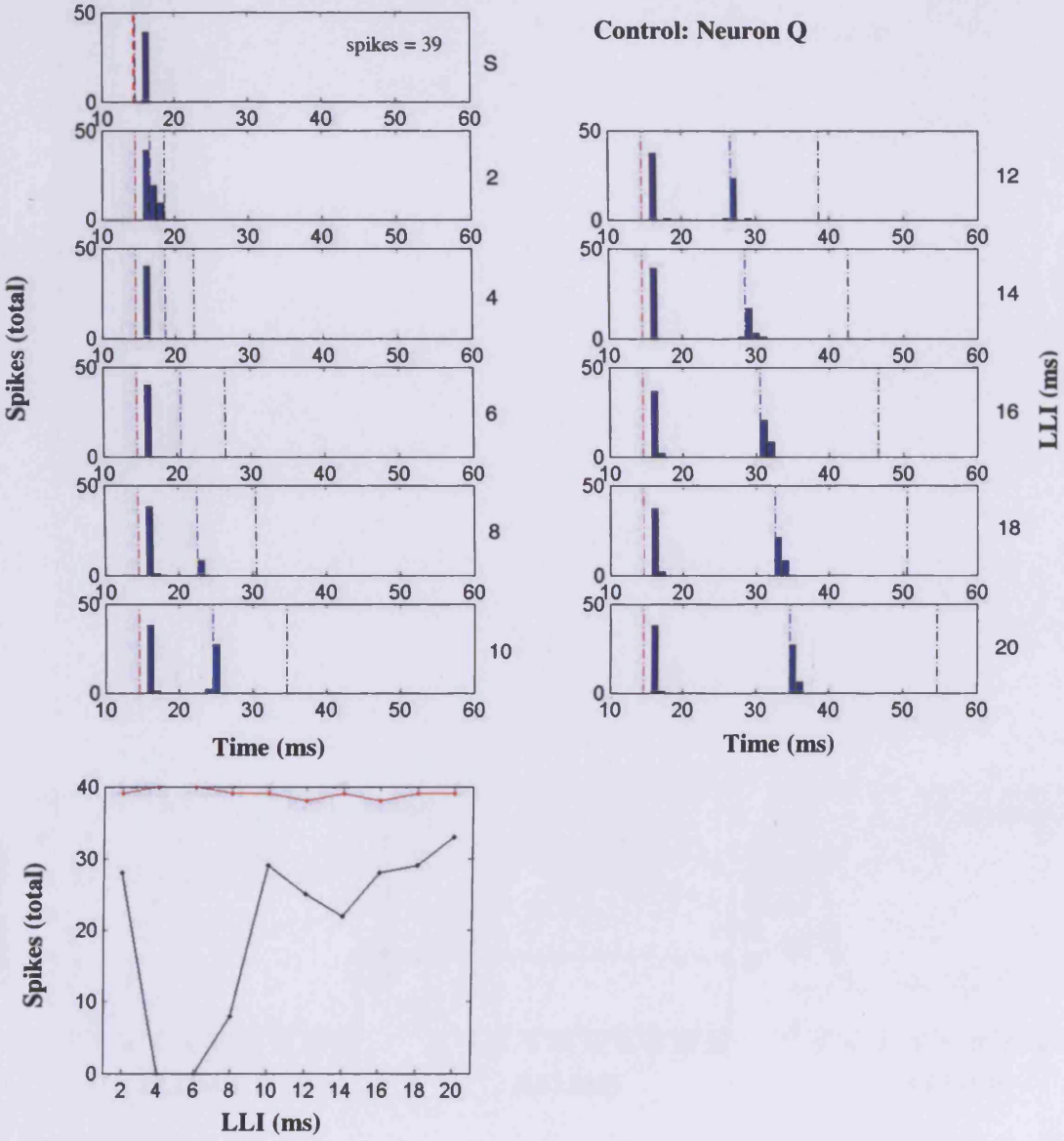


Figure 6.2.5 Post stimulus time histograms of the response of neuron Q to 40 repeats of a single binaural click (S) and binaural click pairs with LLIs of 2-20ms. Bottom panel, the red line represents the total number of spikes to the lead click summed over window from post stimulus onset to the arrival of the lagging click (red dotted line to blue dotted line on PSTHs). The black line represents the adjusted number of spikes in response to the lagging click. Actual calculations were preformed on raw spike times, not binned PSTH spike times.

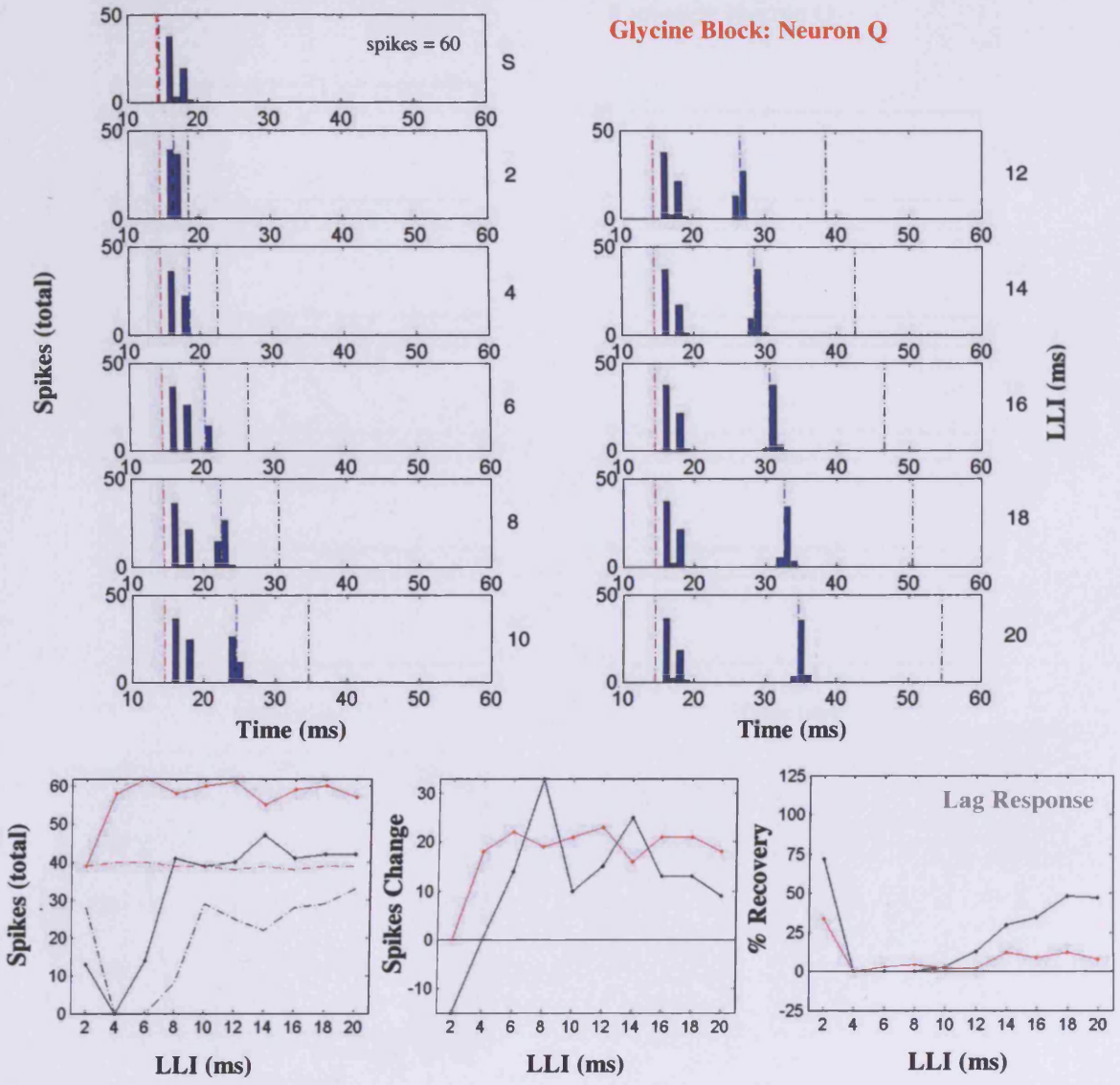


Figure 6.2.6 Post stimulus time histograms of the responses of neuron Q to 40 repeats of a single binaural click (S) and binaural click pairs with LLIs of 2-20ms presented at best ITD at an intensity of 35 dB SPL (Top panels) under application strychnine. Bottom panel, the red line represents the total number of spikes to the lead click summed over window from post stimulus onset to the arrival of the lagging click (red dotted line to blue dotted line on PSTHs). The black line represents the total number of spikes in response to the lagging click. The bottom middle panel shows the difference in spikes evoked by the lead click (red line) and the lag click (black line) in the drug condition compared with the control condition. The bottom right panel shows the lagging click recovery functions for the control (black line) and Glycine block (red line) conditions.

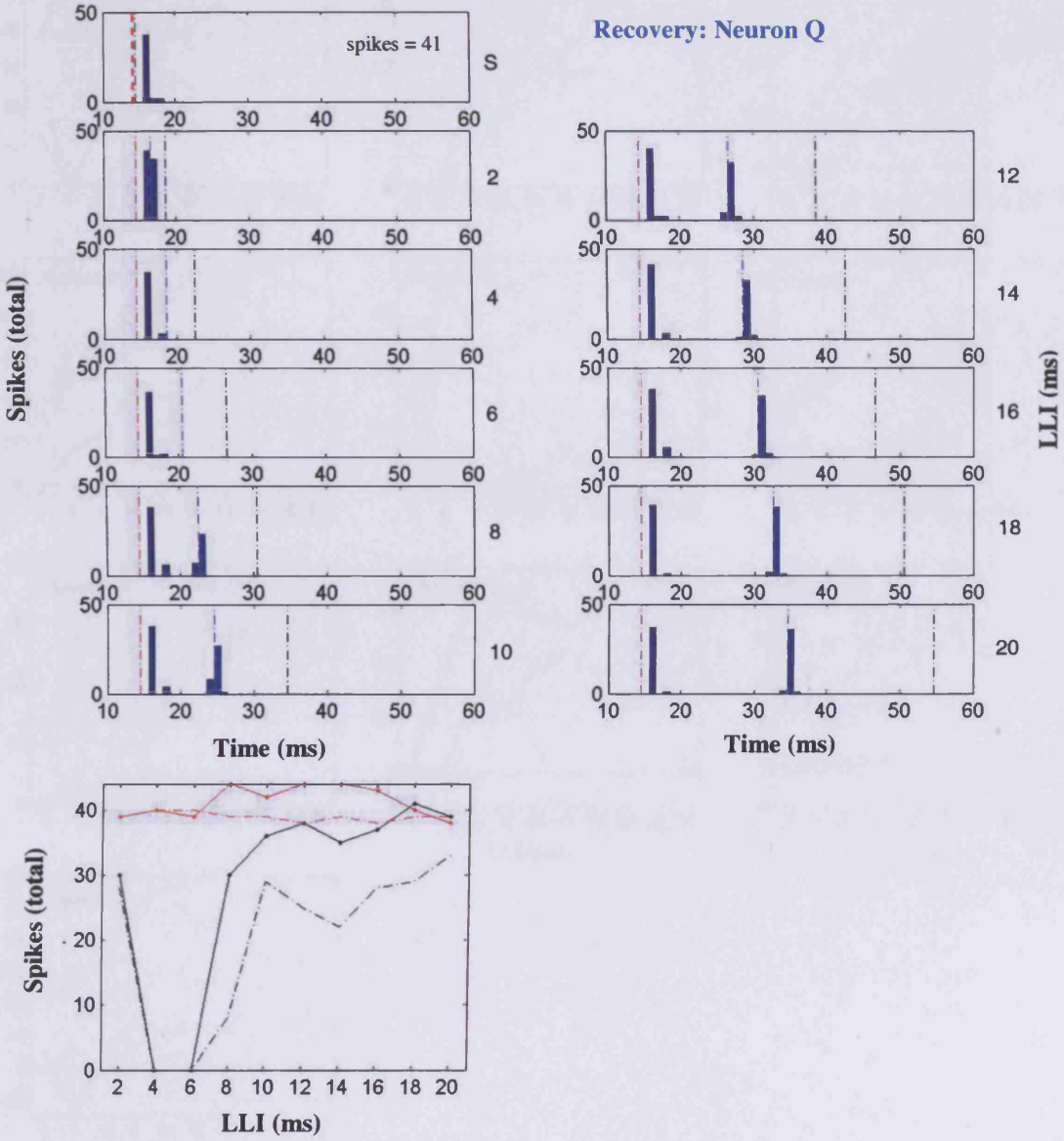


Figure 6.2.7 Post stimulus time histograms of the response of Neuron W to 40 repeats of a single binaural click (S) and binaural click pairs with LLIs of 2-20ms (Top panels), following termination of application of strychnine. Bottom panel, the red solid line represents the number of spikes occurring during the leading click window (red dotted line to blue dotted line on PSTHs). The black solid line represents the adjusted number of spikes occurring during the lagging click window. The dotted lines are the corresponding spike counts from the control condition.

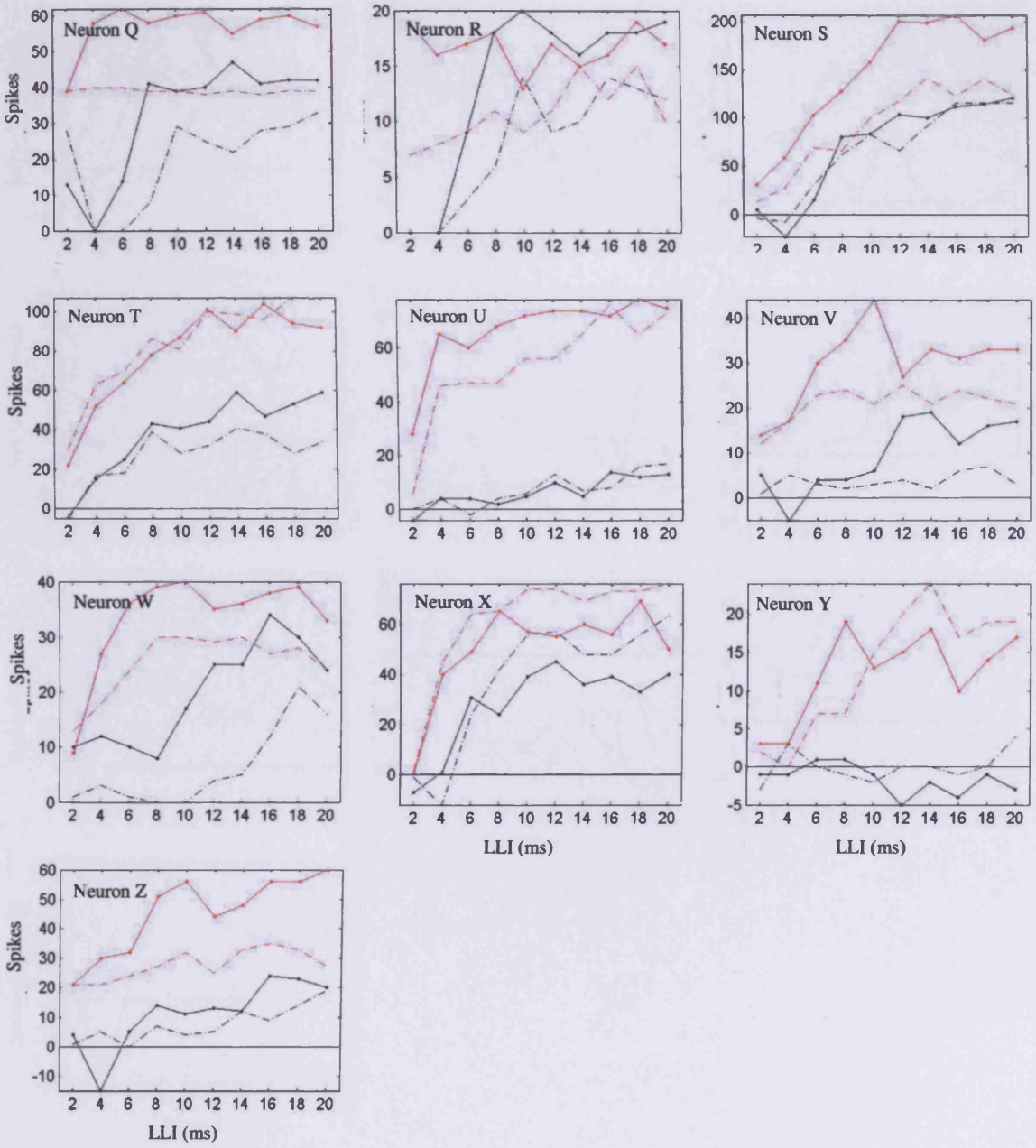


Figure 6.2.8 Number of spikes in response to 40 repeats of a single binaural click and binaural click pairs during application of strychnine. The solid red line represents the number of spikes summed over the leading click window. The solid black line represents the number of spikes summed over the lagging click window. The corresponding dotted lines are responses obtained under the control condition. Responses to lagging clicks have been adjusted for spikes that could be accounted for in responses to the leading click.

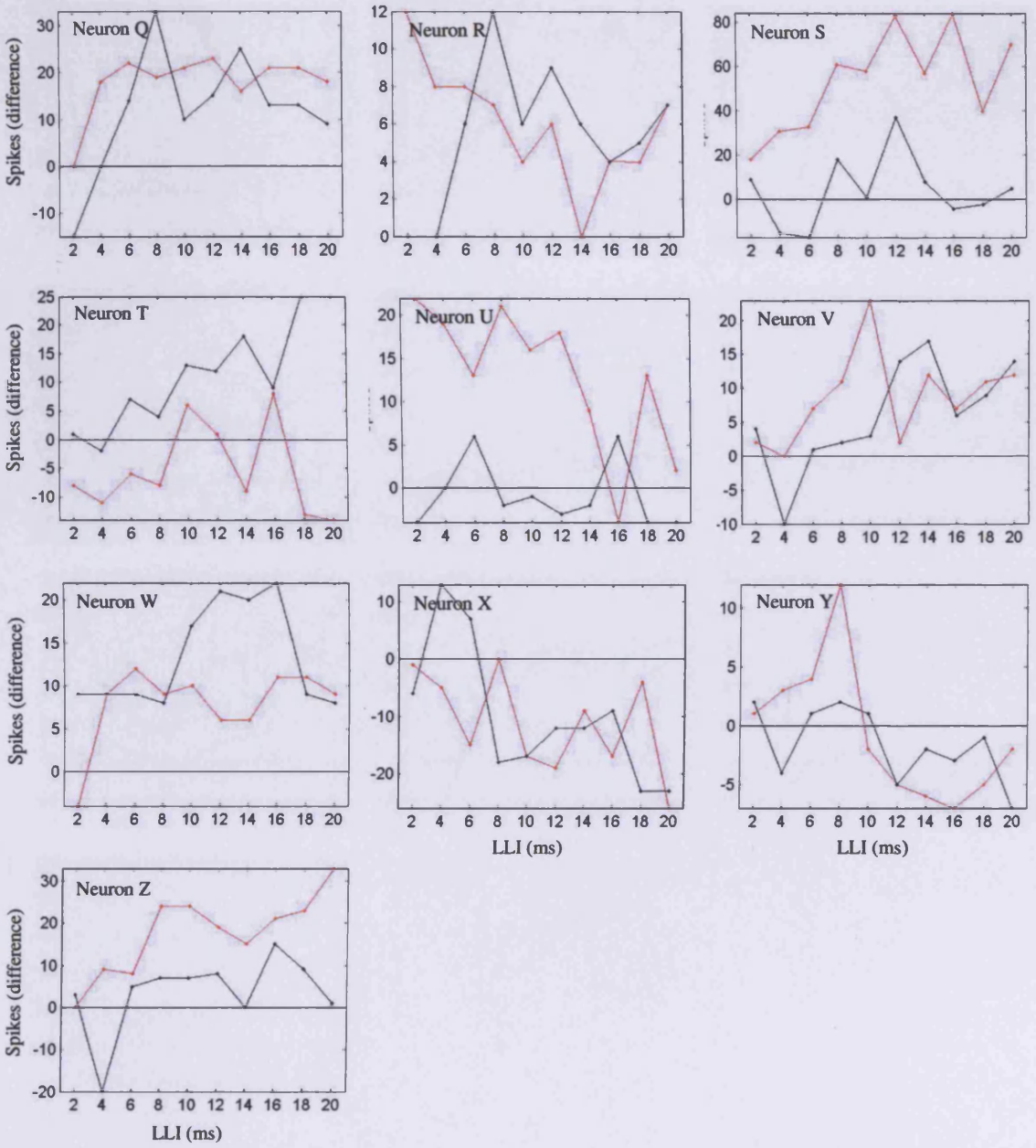


Figure 6.2.9 Difference in the number of spikes in response to 40 repeats of a single binaural click (s), and binaural click pairs separated by LLIs of 2-20ms during application of strychnine and a control condition. The solid red line represents the difference between the leading click window spike counts, the solid black line represents the difference between the lagging click window spike counts. Responses to lagging clicks have been adjusted for spikes that could be accounted for by responses to the leading click.

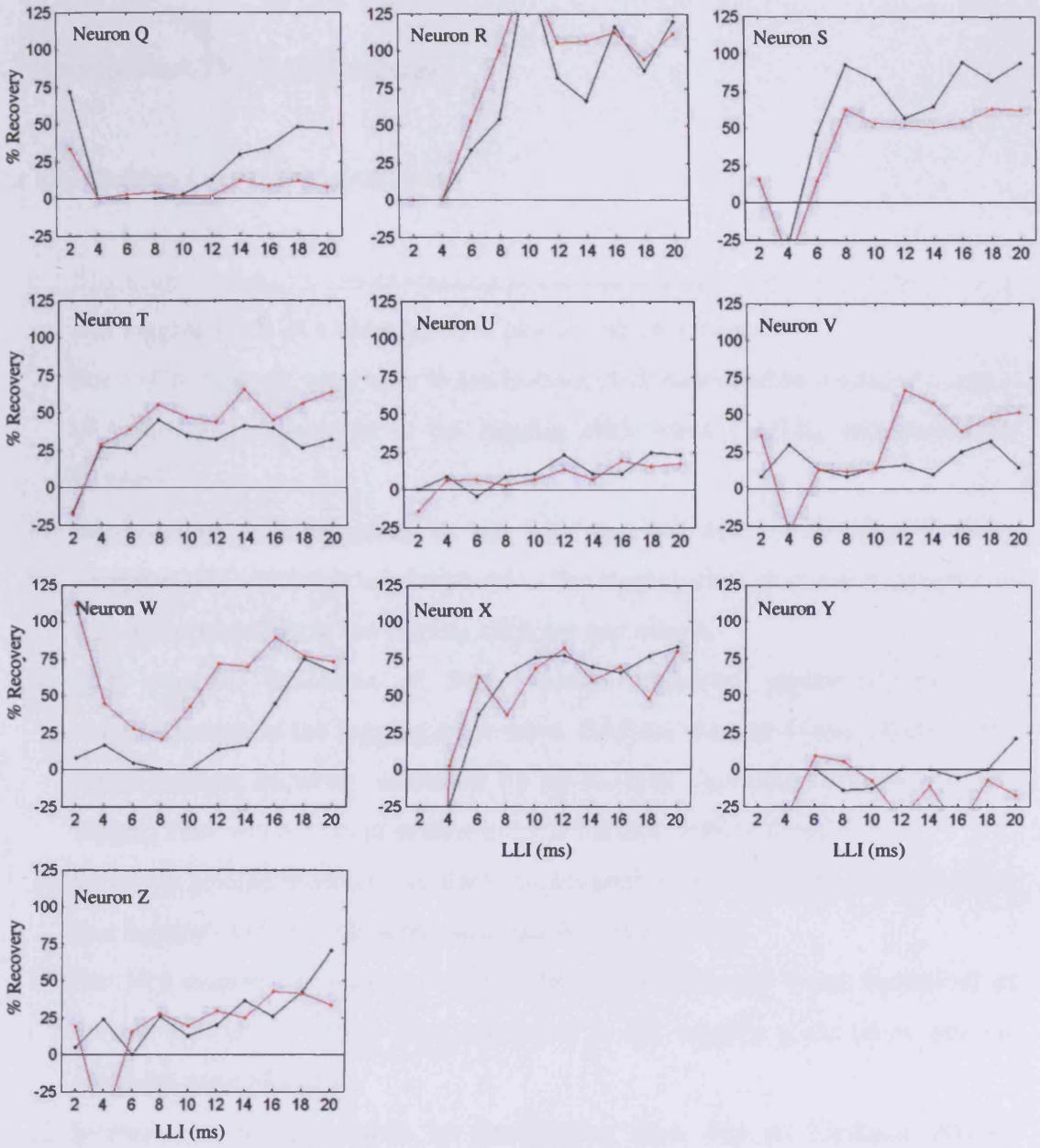


Figure 6.2.10 Lagging click recovery functions for control (black lines) and Glycine block (red lines) conditions.

6.3 Results Part IV: Key Findings

The key findings from this chapter were,

6. Blocking $GABA_A$ receptors resulted in increased neural responses to the leading *and* lagging click of a binaural click pair for 16/16 neurons.
7. For 11/16 neurons, responses to the leading click increased by a greater number of spikes than responses to the lagging click when $GABA_A$ receptors were blocked.
8. Increases in responsiveness to the lagging click due to blocking $GABA_A$ receptors did not result in a response to the lagging click that was equivalent to that of the response to the leading click for any neuron.
9. The recovery functions of 5/16 neurons indicated greater recovery of responsiveness to the lagging click when $GABA_A$ receptors were blocked. For these neurons, recovery increased by up to 45%. However, responses to the lagging click did not occur at shorter LLIs for any of these neurons.
10. Blocking glycine receptors resulted in increased neural responses to the leading *and* lagging click of a binaural click pair for 7/10 neurons.
11. For 3/10 neurons, responses to the leading click increased by an equivalent or greater number of spikes than responses to the lagging click when glycine receptors were blocked.
12. Increases in responsiveness to the lagging click due to blocking glycine receptors did not result in a response to the lagging click that was equivalent to that of the response to the leading click for any neuron.
13. The recovery functions of 3/10 neurons indicated greater recovery of responsiveness to the lagging click when glycine receptors were blocked. For 2 neurons, this resulted in a representation of the lagging click at shorter LLIs.

7.0 Discussion

The precedence effect describes the dominance of the first arriving wave front from a sound source on auditory perception, over reflected copies of that sound arriving milliseconds later. The precedence effect is most apparent during the time over which the direct sound and its reflections are perceived as a single auditory event; a period referred to as fusion. The dominance of the spatial location of the leading sound is referred to as localization dominance. Two alternatives have been proposed to explain the physiological basis of localization dominance which are referred to in this thesis as the neural suppression hypothesis, and the cochlear processing hypothesis.

Localization dominance can be described as the temporal weighting of directional cues conveyed by the leading sound relative to those conveyed by a later arriving copy of that sound. The greater perceptual weight given to the directional information conveyed by the leading sound explains why listeners hear a sound originating from near the location of the leading sound, with a slight bias in localization judgments toward the location of the lagging sound (Wallach et al. 1949). When presented with binaural click pairs representing a direct sound and a single reflection, neurons in the IC of a number of species respond best to the leading click and show a reduced response to the lagging click. This provides a neural mechanism for temporal weighting of directional information conveyed by leading sounds.

The neural suppression hypothesis claims that the neural suppression of lagging clicks that is apparent in the output of IC neurons can account for localization dominance. It has been suggested that convergent GABA-ergic inhibitory input from the DNLL (Yin, 1994; Litovsky & Yin, 1998b; Fitzpatrick at al. 1995; Pollack & Burger, 2002), or glycinergic inhibitory input from the LSO (Fitzpatrick at al. 1995) onto IC neurons, explains the suppression of lagging click responses (see Introduction section 1.2.2 & 1.3).

The cochlear processing hypothesis suggests that binaural click pair stimuli interact in the cochlea resulting in alterations to the binaural directional cues originally conveyed by the leading and lagging clicks of the stimulus (Tollin, 1998; Hartung & Trahiotis

2001). The resulting ITD and IID cues conveyed by the altered internal representation of the leading *and* lagging click explain the behaviour of listeners localising transient stimuli, rather than a weighting of the directional cues conveyed by the leading click (Tollin & Henning 1998; Hartung & Trahiotis 2001). The implications of this for a single IC neuron are such that the neural representation of the leading click would represent directional information conveyed by the leading and lagging clicks (see Introduction section 1.2.3 & 1.3).

The results of the experiments presented in this thesis indicated that the output of neurons in IC was consistent with that predicted by the cochlear processing hypothesis. The results did not support the neural suppression hypothesis.

7.0.1 The Majority of Neurons had Low Best Frequencies and were ITD Sensitive (Results Part I: Section 3.1)

Localization dominance observed in the precedence effect is a sound localization task. Therefore, the neurons selected for experimentation reflect those most likely to be relied upon to determine sound source location by a low-frequency hearing animal such as the guinea pig. The frequency-versus-intensity response areas collected for each neuron confirmed that the population of neurons examined for this thesis was comprised primarily of neurons with low best frequencies (< 2 kHz; 73/108; Figure 3.1.2). Additionally, 67/108 neurons were sensitive to the ITD imposed on a single binaural click. The modulation of responses of neurons sensitive to ITDs were classified according to the shape of their ITD function as belonging to one of four types, peak type, periodic type, trough type or sigmoidal type (Figure 3.1.4), all of which have been reported in IC neurons of the cat in response to tonal and noise stimuli (Geisler, 1969; Carney & Yin, 1989) and in response to binaural clicks (Benevento & Coleman, 1970; Carney & Yin, 1989). Humans, a low-frequency hearing species, are known to rely on directional cues conveyed by low-frequency sound when locating a sound source (Wightman & Kistler, 1992). Therefore, the results obtained from the group of low-BF, ITD sensitive neurons examined in this study should provide a reasonable model of the human auditory system.

7.0.2 The 10-dB Bandwidth of Neurons Increased with Increasing BF (Results Part I: Section 3.1)

The computational modeling of the auditory system implemented in the studies of Tollin (1998) and Hartung and Trahiotis (2001) used auditory filter bandwidths of 1/3 of an octave. Therefore, filters with low CFs had narrower bandwidths, in hertz terms, than those with higher CFs, reflecting the frequency filtering properties of the mammalian auditory system. In both of these studies, the degree of interaction between the leading and lagging clicks predicted by the modeling procedures and as such, the degree of alteration of the directional cues present in the clicks, depended on the bandwidths of the filters. Finding evidence of cochlear processing in the output of IC neurons that was filter bandwidth dependent would provide support that the results

were linked to cochlear processing. In the neurons studied for this thesis there was a progressive broadening of 10-dB bandwidths with increasing BF. As such, it was expected that evidence of BF dependent cochlea processing would be found in the responses of the current sample of neurons.

7.0.3 Neurons Showed Distinct Temporal Patterns in their Responses to Binaural Clicks
(Results Part I: Section 3.2)

Temporal patterns observed in the output of neurons were described and categorised. Most neurons responses to a single binaural click were classified as either a single or multiple response type (Figure 3.2.1). The majority of neurons (55/84) responded with a single and continuous period of spike activity that varied in duration between neurons (single response type). The inter quartile range of the response durations showed a clear BF dependence for neurons with BFs <1 kHz above which the interquartile range indicated neurons response times were relatively homogenous (<2 ms). It was thought that this BF dependence of response duration may reflect the response duration of the cochlea. When stimulated with a transient which causes the cochlea to resonate low-BF neurons of the auditory nerve (AN) respond for a longer duration than high-BF neurons of the AN (Lin & Guinan Jr., 2000). However, the responses in the AN are also periodic as a result of phase locking, a characteristic not observed in this study for neurons that would also be capable of phase locking (BFs < 600 Hz). Nevertheless, a BF dependence of response duration when presented with a click has not previously been observed in neurons of the IC with several studies reporting the response time of neurons of the IC to be unrelated to their BF (Fitzpatrick et al. 1995; Yin, 1995).

The second most common response to a single binaural click was the multiple response type. These neurons exhibited multiple periods of spiking activity in response to a single binaural click (23/84 neurons). This was not related to the periodicity of the neuron (1/BF), the intensity of the stimulus or the ITD of the stimulus, ruling out filter dependent peripheral effects, an artefact due to an individual response to each monaural click, or an offset response due to the intensity of the stimulus. Multiple periods of firing have been reported in neurons of the IC of the cat (Benevento & Coleman, 1970) and are apparent in the data from other studies using clicks as stimuli (Yin, 1994; Litovsky & Yin 1998a; Litovsky, 1998).

7.0.4 Responses of Neurons to the Leading Click of a Binaural Click Pair Delivered with the Same ITD were Modulated as a Function of the LLI

(Results Part II: Section 4.1)

The results supported the hypothesis that for binaural click pairs delivered with the same ITD, the lagging click of a binaural click pair would either suppress or enhance a neurons response to the leading click depending on the LLI of the stimulus (Hypothesis 1, part 1; Introduction, section 1.4.). The hypothesis that the enhancive or suppressive effect of the lagging click would be greater for low-BF neurons than for high-BF neurons was not supported (Hypothesis 1, part 2; Introduction, section 1.4.). Therefore, previous findings suggesting leading click responses to be independent of lagging click responses are not supported by the results of this thesis, although there was no indication that this was the result of cochlear processing.

The responses of the majority (55/80) of neurons to the leading click of a binaural click pair both delivered at best ITD were modulated by the lagging click by greater than 30% of their response to a single binaural click (Figure 4.1.5). The modulation of the response for many neurons also depended on the lead-lag interval (LLI) between the clicks. The effect of the lagging click on neural responses to the leading click was *either* suppressive or enhancive (Figures 4.1.5-4.1.6). For some neurons, whether the effect was suppressive or enhancive depended on the lead-lag interval (LLI) of the stimulus (Enhance/Suppress type neurons; Figure 4.1.5-4.1.6).

This finding has not previously been reported although it is apparent in the data of Fitzpatrick et al. (1995). Neuron A, in Figure 3, of Fitzpatrick et al. (1995) shows impressive variability in its response to the leading click as a function of the LLI as does neuron A in Figure 5. If it is present in the results of others, it presents a problem for studies that have utilised an estimate of the response to the leading click to calculate recovery functions for LLIs where response windows overlap (see Methods section 2.5). The accuracy of this method relies on the response to the leading click to be constant as a function of the LLI a condition clearly not met by many of the neurons in this study. Tollin et al. (2004) claim that previous studies (Yin, 1994; Fitzpartrick, 1995; Parham et al. 1996; Wickesberg & Stevens 1996; Litovsky & Yin, 1998a,b) have assumed the response to the leading click to be unaffected by the response to the lagging click, using this as a justification for their method of calculating recovery

functions. However, none of these studies have qualified this claim with analysis. This is considered further later in the discussion.

The response to the leading click being modulated by the lagging click is predicted by the cochlear processing hypothesis of localization dominance. The cochlear processing hypothesis would also predict that the modulation of the leading click response would be dependent on the BF of the neuron and that LLI dependence of the effect would be related to the periodicity of the neuron. Assuming that processing of binaural click pairs in each ear is equivalent, cochlear processing of binaural click pairs delivered with the same ITD should not result in alteration to the ITD or IID information conveyed by the stimulus. Because the interval between the click pairs in each ear is the same when the ITD is the same, enhancement or suppression of neural responses to the leading click should depend solely on the phase relationship between the response to the leading click with the time of arrival of the lagging click. Therefore, any variations in the response of neurons to the leading click will not be determined by their ITD or IID sensitivity making the results comparable across neurons of different ITD and IID tuning characteristics. For IC neurons, the periodicity of the response to the leading click should be determined by the neurons BF (1/BF) if their BF reflects the BF of their inputs from the cochlea. Additionally, the low-frequency region of the cochlea will respond for a longer duration than the high-frequency region, and neurons that receive low-frequency input from the periphery will be affected by cochlear processing for a longer duration than neurons receiving high frequency input This is the case in the auditory nerve (Lin & Guinan Jr., 2000). Therefore, if the observed alterations to the neural response to the leading click were due to cochlear processing it would be expected that a) neurons with low-BFs would exhibit greater enhancive or suppressive effects and for a longer duration than neurons with high BFs, and b) the suppressive or enhancive effect of the lagging click should reflect the periodicity of the neuron.

In the current sample of neurons the magnitude of enhancement or suppression when averaged across LLI was not predicted by BF (Figure 4.1.6). As the increments in LLI used in the current study were 1 ms it is not possible to conclude from the results whether the enhancement or suppression of responses to the leading click were predicted by the periodicity of the neurons. However, for neurons with BFs near 1 kHz, the lagging click should always either enhance or suppress the response to the leading

click as the periodicity of such neurons is 1ms. However, neurons with BFs distributed around 1 kHz and separated by less than 100 Hz showed opposite effects of the lagging click i.e. enhancement compared with suppression (Figure 4.1.7) This provides tentative evidence that the effect of the lagging click was not predicted by phasic interactions determined by the neurons BF.

The failure to find a relationship between neuronal BF and the magnitude of the effect caused by the lagging click on responses to the leading click may have been due to a sampling bias in the data toward neurons with lower BFs (68% had BFs < 2 kHz). This bias would mean that the neurons examined may have received inputs from similar frequency regions of the cochlea and as such exhibited effects of the lagging click that were of a similar magnitude. It is also possible that such effects could be the result of relatively long temporal integration of inputs from lower auditory nuclei in neurons for which enhancement or suppression of leading click responses was observed. The model of Hartung and Trahiotis (2001) used a temporal integration window of 30 ms, and Tollin (1998) a window of 5 ms, indicating this may be the important factor in the success of these models to predict the outcomes of localization dominance studies. Regardless of the origin of the phenomenon, it was clear from the results of this thesis that neural responses to the leading click of a binaural click pair were affected by the lagging click in the majority of neurons examined. This finding has not previously been reported by studies of the precedence effect in IC neurons.

7.0.5 Responses to the Leading Click of a Binaural Click Pair are Modulated as a Function of the ITD of the Lagging Click

(Results Part III: Section 5.1)

The results supported the hypothesis that for a particular LLI a neuron's response to the leading click of a binaural click pair would be either suppressed or enhanced depending on the ITD of the lagging click (Hypothesis 2; Introduction section 1.4). The results did not support the hypothesis that any observed suppressive or enhancive effects of the lagging click of a binaural click pair on neural response to the leading click would be greater for more intense stimuli (Hypothesis 3; Introduction, section 1.4). The support for the first hypothesis indicates that the responses of low-BF, ITD sensitive, IC neurons are predicted by the cochlear processing hypothesis.

Responses of low-BF, ITD sensitive neurons to the leading click of a binaural click pair presented at a particular LLI were modulated depending on the ITD imposed on the lagging click. The pattern of modulation was consistent with the same neurons response to a single binaural click presented with the same ITD as the lagging click (Figure 5.1.1-5.1.6). For many neurons, the degree of modulation of their response to the leading click of a click pair not only followed the pattern predicted by the ITD of the lagging click but, was also equivalent in magnitude to that of their ITD function (Neurons A-C; Figures 5.1.1-5.1.6). Therefore, the portion of a neuron's response prior to arrival of the lagging click represented directional information from both the leading *and* lagging clicks and in some cases with the same resolution as the neuron's response to a single binaural click presented with the same ITD. For the majority of neurons reducing the stimulus intensity by 15 dB, or to response threshold, had little effect on the effect on the modulation of responses to the leading click (Figures 5.1.9-5.1.0). For the two example neurons shown for which a reduction in stimulus intensity did affect the modulation of the leading click response, for Neuron D (Figures 5.2.1-5.2.4) it decreased it, and for neuron E (Figures 5.2.5-5.2.8) it increased it.

Yin (1994), reported that when presented with binaural click pairs separated by an LLI of 1 ms that neurons in the IC of the cat responded to an ITD that was between that conveyed by the leading and lagging clicks. For longer LLIs there was no such effect reported. Yin (1994) interpreted this finding as a neural correlate of summing localization. This is supported by evidence of neural correlates of summing localization found in auditory neurons of the owl (Keller & Takahashi, 1996). The results from the current study indicate that in low-BF IC neurons "summing localization" extends at least up to 5 ms suggesting that this effect is not related to summing localization, which occurs for LLIs of up to 1 ms for binaural click pairs.

The neural suppression hypothesis suggests that localization dominance occurs due to temporal weighting of the directional cues conveyed by the leading click. The physiological basis of this is a neural representation of the leading click that is more robust than that of the lagging click. Therefore, listeners' localization judgments are influenced to a greater degree by the directional cues present in the leading click. In such a model, the processing of the leading click occurs prior to, and independently to, that of the lagging click i.e. processing is sequential. The low-BF, ITD sensitive

neurons in the current study however did not process binaural click pairs in the sequential fashion suggested by a temporal weighting model of localization dominance (Yin, 1994; Litovsky & Yin, 1998, a, b) as indicated by the influence of the lagging click ITD on neural responses to the leading click. Rather, the neurons in this study represented directional information present in both the leading and lagging click in their response to the leading click and presumably in their response to the lagging click. This is consistent with the suggestion of Hartung and Trahiotis (2001) that cochlear processing of low-frequency sound alters the directional cues conveyed by the leading click in such a way that they are influenced towards the directional information conveyed by the lagging click. Therefore the responses of low-BF, ITD sensitive, neurons for LLIs of 2-5 ms which are known to result in localization dominance for humans when presented with binaural click pairs over headphones (Wallach et al. 1949; Ebata et al. 1968; Freyman et al. 1991; Yang & Grantham, 1997a, 1997b; Litovsky et al. 1999), can explain the localization dominance of the leading click without the requirement of neural suppression necessary to the temporal weighting model.

While it is clear that the neurons in this study 'behaved' in accordance with the model described by Hartung and Trahiotis (2001), attempts to connect the phenomenon with cochlear processing were not conclusive. It was expected that higher stimulus intensities would result in a response of longer duration in the cochlea (Hartung & Trahiotis, 2001) and as such, there would be a greater effect of the lagging click ITD on neural processing of the leading click. Reducing the intensity of the stimulus however had little effect on the modulation of neurons responses to the leading click as a function of the lagging click ITD for the majority of neurons. While the effect of the lagging click was small for these neurons, a reduction in stimulus intensity increased rather than decreased the effect of the lagging click. This is not predicted by the cochlear processing hypothesis that suggests that lower stimulus intensities result in a shorter cochlear response to the leading click that is of a smaller magnitude than high intensities. As such, there is less interaction between the response to the leading click and that to the lagging click.

Stimulus intensity may not have affected responses of the low-BF neurons in the sample as they did not respond over a broad range of intensities and as such, changes in the intensity of the stimulus were generally restricted to between 15 and 25 dB peak

SPL. This reduction in intensity may not have been sufficient to notice a reliable change in the modulation of the leading click response in many neurons. For the individual examples presented in section 5.2 for which there was a greater effect of stimulus intensity, one neuron showed a decrease in modulation (Neuron D; Section 5.2) while the other showed an increase (Neuron E; Section 5.2). The lack of robust effects of stimulus intensity and the observation of contradictory effects make it difficult to draw a conclusion concerning the effect of stimulus intensity on the effect of the lagging click on neural responses to the leading click. The question of whether modulation of leading click responses as a function of the lagging click ITD is related to cochlear processing therefore remains open.

Regardless of the mechanism behind modulation of the leading click response, the output of low-BF, ITD sensitive neurons in response to binaural click pairs was predicted by the cochlea processing model proposed by Hartung and Trahiotis (2001).

7.0.6 Responses to the Lagging Click of a Binaural Click Pair were Suppressed by the Leading Click in All Neurons (Results Part II: Section 4.1)

Responses to the lagging click of a binaural click pair were suppressed by neural responses to the leading click of a binaural click pair for all neurons examined (Figure 4.1.9). Describing the time course of recovery of neural responses to the lagging click was not an aim of this thesis. As such, neurons were presented with stimuli for which LLIs were relevant to behavioural measures of localization dominance rather than longer LLIs aimed at describing recovery. The group of neurons used to explore the effects of inhibitory neurotransmitters on the processing of binaural click pairs were presented with the longest LLIs (up to 20 ms) and, as such, are more comparable with those of previous studies.

For the group of neurons presented with comparable LLIs to previous studies (group A, 2-20 ms) neurones exhibited 50 % recovery (calculated using non-overlapping response windows) for an LLI of 16 ms, 6.2 ms longer than for IC neurons reported in the un-anaesthetised cat (9.8 ms; Tollin et al. 2004), 4.1 ms longer than in the un-anaesthetised rabbit (11.9 ms; Fitzpatrick et al. 1995) and 18.8 ms earlier than reported in barbiturate anaesthetised cat (34.8 ms; Litovsky & Yin 1998a). It is surprising that the neurons in

the current study exhibited 50% recovery times that were more comparable with the un-anesthetised preparations than with the anaesthetised preparations reported by Tollin et al. (2004). This may be due to differences in the anaesthetic used or the BFs of the neurons comprising the sample. Tollin et al. (2004) described comparable 50% recovery times for IC neurons in the un-anaesthetised cat and un-anaesthetised rabbit but not between the anaesthetised cat and un-anaesthetised rabbit. This suggests that anaesthesia rather than species differences account for major differences in the 50 % recovery time of neural responses to lagging clicks.

Lagging click recovery functions calculated according to the method described by Tollin et al. (1994) as well as those calculated only for LLIs where the leading and lagging click responses windows did not overlap were presented in the results (Figure 4.1.9 & 4.1.10). Using an estimate of a neurons response to the leading click to calculate recovery functions for LLIs where neural responses to the leading and lagging click were not discrete (Tollin et al. 2004) however proved ineffective for this population of neurons. This strategy resulted in estimates of recovery of responsiveness to the lagging click that indicated greater recovery for short LLIs that declined as the LLI increased (Figure 4.1.10). For the current sample of neurons, this was largely the result of there being a single continuous response to the stimulus despite there being a leading and lagging binaural click due to the short LLIs examined (see Neuron B; Figure 6.2.5). In these instances, a binaural click pair often evoked a greater response than a single binaural click; however, the increased response did not necessarily occur timed with, or as an obvious representation of the lagging click. It is questionable then whether the neuron was responding to the lagging click per se or its spike count simply increased due to the additional acoustic energy afforded by the lagging click. This situation was exemplified by neuron B in section 4.1 of the results (Figures 6.1.5-6.1.7). Recovery functions generated using only stimulus conditions for which there was no overlap in neural response to the leading and lagging clicks gave an estimate of recovery that increased with increasing LLI and, as such, gave more reliable estimate of recovery from suppression.

Previous studies of the precedence effect have not focused on short LLIs and as such recovery functions most probably provide an accurate estimate of responsiveness of neurons to the lagging click. However, recovery functions for LLIs where response

windows overlap do not necessarily describe the recovery of a discrete response to the lagging click. Rather, they describe whether there has been an increase in the number of evoked spikes as a result of the stimulus comprising two binaural clicks as opposed to one (neuron B, Figures 6.1.5-6.1.7). Likewise, the recovery of a group of neurons derived from recovery functions describes whether there was a greater number of spikes evoked by a binaural click pair and not whether a response to the lagging click, discrete to that of the leading click, recovered. While this is not necessarily problematic, it places limitations on the interpretation of experiments describing the recovery of responses to the lagging click that do not additionally explain whether the response to the lagging click was discrete from that to the leading click.

7.0.7 Inhibitory Neurotransmitters were not Responsible for the Suppression of Lagging Click Responses in Most Neurons

(Results Part IV: Section 6.1)

The results provided limited support for the hypothesis that blocking GABA-ergic inhibition with the $GABA_A$ receptor antagonist gabazine would result in a decrease in the suppressive effect of the leading click of a binaural click pair on neural responses to the lagging click (Hypothesis 4; Introduction, section 1.4). The results also provided limited support for the hypothesis that blocking glycinergic inhibition with the glycine receptor antagonist strychnine would result in a decrease in the suppressive effect of the leading click of a binaural click pair on neural responses to the lagging click. The limited support for these two hypotheses was not sufficient to support the neural suppression hypothesis of localization dominance as the majority of neurons did not show increases in responsiveness to the lagging click when either $GABA_A$ or glycine receptors were blocked. Additionally, the increases in recovery observed in some neurons increased with increasing LLIs rather than decreased as would be necessary to explain localization dominance.

Responses of neurons examined in this thesis indicated that blocking GABA-ergic inhibition with the $GABA_A$ receptor antagonist gabazine did not decrease the suppressive effect of the leading click on neural responses to the lagging click for most neurons (69%; Figure 6.1.13, neurons A, B, C, E, F, G, H, I, J, N, & O). While blocking $GABA_A$ receptors resulted in an increase in responsiveness to binaural click

pairs this could be explained primarily by an increase in the number of spikes evoked by the leading binaural click of the binaural click pair (Figure 6.1.11). GABA-ergic inhibition for these neurons arrived simultaneously with the excitation in response to binaural click pairs and for most neurons was proportional to the excitation evoked by the response to each click i.e. inhibition was strongest for the response to the leading click which evoked a greater number of spikes. Therefore, for most neurons examined (69%), GABA suppressed the response to the leading click more than to the lagging click effectively emphasising responses to the lagging click. This is the opposite of the effect predicted by the neural suppression hypothesis.

For 31% of the neurons examined there was an increase in responsiveness to the lagging click as a result of blocking $GABA_A$ receptors (Figure 6.1.13; neurons D, K, L, M, & P). For these neurons, increases occurred only where there was a response to the lagging click present in the control condition. Therefore, GABA did not decrease the LLI at which the neuron responded to the lagging click as would be expected if it mediated echo threshold. However, for these neurons, GABA-ergic inhibition decreased the magnitude of the response to the lagging click and by doing so emphasised the response to the leading click in a manner consistent with temporal weighting and the neural suppression hypothesis. Despite the increase in lagging click recovery seen in these neurons, the response to the leading click was greater than that to the lagging click in both the control condition and the GABA block condition for all lead-lag intervals (LLIs).

These results do not support those of Zhou and Jen (2003) that indicated a decrease in recovery times for the majority of neurons examined in the IC of the big brown bat as a result of blocking $GABA_A$ receptors with bicuculline methiodide. Rather, GABA decreased recovery of response to lagging clicks for very few neurons. Zhou and Jen (2004) proposed that the time course of GABA-ergic inhibition seen in IC neurons of the big brown bat was related to specific requirements of hunting using echo-location. As such, the results from the big brown bat may not be related to the precedence effect. The differences in the requirements of the auditory systems of each species may explain the observed difference between the studies.

As for GABA, responses of neurons examined in this thesis indicated that blocking glycinergic inhibition with the glycine receptor antagonist strychnine did not decrease the suppressive effect of the leading click on neural responses to the lagging click for most neurons (70%; Figure 6.2.10, neurons Q, S, U, V, Y, X & Z). Blocking glycine receptors with strychnine resulted in a decrease in the suppressive effect of the leading click on neural responses to the lagging click for only 3/10 neurons (Figure 6.2.10, neurons T, W & R). Both neurons had high BFs (> 8 kHz). For 10 of the 26 neurons examined, blocking glycine resulted in an increase in responsiveness to binaural click pairs which was, for most neurons, was the result of either a greater increase in responsiveness to the leading click or an equivalent increase in responsiveness to the leading and lagging click of a binaural click pair (Figures 6.2.8-6.2.9). For the two neurons for which blocking glycine resulted in a decrease in suppression of the response to the lagging click, the greatest release from suppression occurred for LLIs greater than 12 ms. For none of the neurons examined did blocking glycine receptors result in a response to the lagging click that was equivalent to that of the leading click.

These findings support the proposal by Fitzpatrick et al. (1995) that glycinergic inhibition originating in neurons of the LSO may suppress neural responses to the lagging click of a binaural click pair in IC neurons. However, given the limited number of neurons that showed decreased suppression of lagging click responses, it is unlikely that glycinergic inhibition forms the basis of localization dominance.

Neither the suppressive effect of GABA-ergic or glycinergic inhibitory input to IC neurons accounted for all of the suppression of lagging click responses in these neurons. For most neurons (73%), the result of blocking either neurotransmitter did not increase recovery of responses to the lagging click beyond 50% (Figure 6.1.13 & 6.2.10). As such, much of the suppression of the lagging click responses apparent in the responses of IC neurons in the guinea pig appears to be the result of auditory processing in lower nuclei. The findings of previous studies showing neural inhibition to be responsible for the suppression of responses to lagging sounds in the IC of the bat (Pollack & Burger 1998; Klug et al. 2002; Zhou & Jen, 2003) and that suggest a similar possibility in IC neurons of the rat (Kelly & Kidd, 2000) do not seem to generalise to IC neurons of the guinea pig which relies on low frequency directional cues to localise sound.

8.0 Conclusion

The two key findings of this thesis are,

1). Low-BF, IC neurons of the guinea pig represent an ITD that is a combination of the ITD conveyed by the leading and lagging click of a binaural click pair in their response to the leading click.

2). GABA-ergic and glycinergic inhibition are not responsible for the suppression of neural responses to the lagging click of a binaural click pair in the majority of IC neurons in the guinea pig. For most neurons the effect of both GABA-ergic and glycinergic inhibition is to suppress responses to the leading click more than responses to the lagging click.

The combined results of this thesis support a model of localization dominance that incorporates integration of auditory responses to the leading and lagging click of a binaural click pair in IC neurons in a manner analogous to that outlined in the cochlear processing model of the precedence effect proposed by Hartung and Trahiotis (2001). Whether this occurs at the level of the cochlea or in neurons located in higher auditory nuclei remains unclear. Although, if integration of directional cues conveyed by the leading and lagging sounds were purely a neural phenomenon it would be expected that localization dominance would operate for all sounds, and not only sounds containing transients. Nevertheless, directional information represented in the responses of low frequency neurons is different to that originally conveyed by the stimulus. The cues are altered in such a way as to bias the neural representation of sound source location in the direction indicated by the lagging click. If this is due to cochlear processing, high-BF neurons would not subject to the same effect as their inputs are derived from auditory filters with broader bandwidths that should be less affected by cochlear processing and as such convey directional information consistent with that present in the stimulus. Listeners localization judgments based on such information would therefore be biased in the direction of the lagging click, even if they relied only on the directional cues present in the neural responses to the leading click ignoring those in the lagging click. The results suggest that the listener would in fact be forced to rely solely upon

directional cues conveyed by the neural response to the leading click. While the results indicated that for the majority of neurons, inhibitory neurotransmitters had the consequence of enhancing the response to the lagging click rather than suppressing it at the level of the IC. Responses to the leading click remained substantially more robust than those to the lagging click with responses to the lagging click being almost entirely absent for LLIs of less than 5 ms.

Localization dominance for low-frequency hearing animals when localizing binaural click pairs is therefore due to low-BF neurons indicating a direction in between that of conveyed by the leading and lagging click in their response to the leading click. The suppression of lagging click responses ensures that responses to the leading click are utilised to locate the stimulus. When considered across neurons of all BFs the location indicated by the response to the leading click is that conveyed by the leading click biased in the direction indicated by the lagging click.

Future Research

The current study did not provide direct support for a cochlear processing model of localization and further research is necessary to determine whether the modulation of responses to the leading click of a binaural click pair originated in the cochlea. Presenting binaural click pairs diotically at intervals that are less than ¼ the duration of the periodicity of a neuron may provide such support. By presenting binaural click pairs with much shorter intervals it may be possible to observe a periodicity in the suppression and enhancement of the response to the leading click that is linked to the periodicity of its BF. If enhancement occurred when the lagging click arrived in phase with the continuing response of a given cochlear filter to the leading click and suppression when the relationship was anti-phasic this would suggest the phenomenon originated in the cochlea. Additionally, it would be possible to establish the magnitude of the effect as a function of neural BF. It is also not clear whether the findings from the current study apply to stimuli of longer duration. Further research is needed to determine whether the effect noticed in the current study has greater applicability to real-world listening environments.

List of Abbreviations

AC	auditory cortex
AN	auditory nerve
ANF	auditory nerve fibre
AVCN	anteroventral cochlear nucleus
BF	best frequency
CF	centre frequency
CN	cochlear nucleus
dB	decibel
DCN	dorsal cochlear nucleus
DNLL	dorsal nucleus of lateral lemniscus
GABA	gamma amino butyric acid
IC	inferior colliculus
ICc	central nucleus of the IC
ICp	peri-central nucleus of the IC
ICx	external nucleus of the IC
IHC	inner-hair cell
IID	interaural intensity difference
ITD	interaural time difference
LLI	lead lag interval
LNTB	lateral nucleus of the trapezoid body
LSO	lateral superior olive
MNTB	medial nucleus of the trapezoid body
MSO	medial superior olive
OHC	outer hair cell
PSTH	post stimulus time histogram
PVCN	posteroventral cochlear nucleus
SOC	superior olivary complex
VCN	ventral cochlear nucleus
VNLL	ventral nucleus of lateral lemniscus

Appendix A

Cues to Sounds Source Location

Cues to Sound Source Location

The ability to detect and locate the source of a sound is made possible, or at the very least effective, by the presence of an ear on each side of the head. The process of binaural, or "two-eared", hearing allows for comparison of information from two different locations in space. As a result, the characteristics of the sound in each ear differ, providing additional information to that available at each ear alone. Comparison of information from either ear often clarifies confusing information in noisy environments and provides the necessary cues to locate accurately the source of a sound.

Auditory cues arising from the interaural comparison of sound are referred to as binaural cues, whilst those available at either ear alone are referred to as monaural cues. Sound localization is most accurate when both binaural and monaural cues are available to the listener, as each type of cue provides complimentary information. Binaural cues provide accurate information concerning the azimuth of a sound source but ambiguous information concerning the elevation of a sound source, and whether the source is located in front of or behind the listener. Monaural cues on the other hand provide little information concerning the azimuth of a sound source, but can resolve the ambiguities present in the binaural cues (Martin et al. 2004).

For a given sound source location there is a difference between the path length a sound has to travel to reach the left and right ears of a listener. This difference results in a difference in the time of arrival of the sound at each ear, referred to as the interaural time difference (ITD). The difference in arrival time at each ear means there is a difference in the onset of the sound at each ear, and for ongoing sounds, a difference in the phase of the wave form at each ear. For ongoing sounds, the ITD is only interpretable for sound frequencies for which the length of one cycle is longer than the distance between the two ears. The interaural phase difference (IPD) in this instance relates to a specific time difference associated with the wavelength of the sound. For frequencies where there is more than one cycle between the ears the IPD is ambiguous. As the location of a sound source changes so too the ITD changes, providing a potent cue to sound source location (Figure A.1).

The solid nature of the head provides a reflective surface for sound. Accordingly, sound will be reflected away from the surface of the head closest to the sound-source, resulting in the sound reaching the ear farthest from the source being less intense than that reaching the near ear. The difference in intensity between the near and far ears is known as the interaural intensity difference (IID) and, like the ITD, is dependent on the angle of incidence of a sound with the head (Figure A.2).The IID caused by the head shadow is greatest for high-frequency sounds and, as such, the IID cue is most useful for high frequency sound localization.

Sound localization in humans is dominated by the ITD cue for sounds containing frequencies below 2.5-kHz and by the IID cue for sounds containing only frequencies above ~ 5-kHz (Wightman & Kistler, 1992). Localization of low-frequency sounds utilising ITD cues, and of high-frequency sounds utilising IID cues, is referred to as the duplex theory of sound localization (Raleigh, 1907).

The ITD and IID cues resulting from a sound source do not vary greatly with changes in the elevation of the sound source, or whether it is in front of or behind the listener. This means that any combination of ITDs and IIDs provides ambiguous information concerning the elevation and front-back location of a sound. This ambiguity is resolved by making use of monaural "spectral cues" (Kuhn, 1979) that result from the interaction of sound, particularly at high frequencies, with the outer ears (pinnae). Reflections created by the convolutions of the pinnae produce location-dependent and frequency-specific changes in the intensity of the sound spectrum (Figure A.3). While monaural spectral cues can be used to determine the elevation and front-back location of a sound in the presence of binaural cues listeners relying solely on monaural spectral cues to localise sound perform little better than guessing emphasising the importance of interaural cues (Martin et al. 2004).

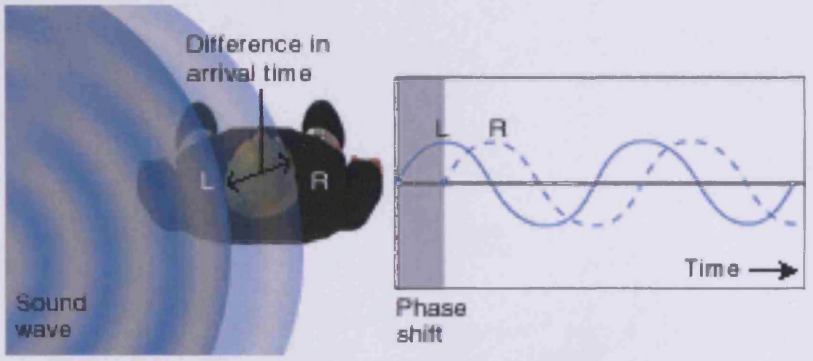


Figure A.1 Differences in the time of arrival of a wave front at the left and right ear referred to as the interaural time difference (ITD) results in an onset difference and an ongoing phase difference (www.brainconnection.com, Scientific Learning Corporation, 1999).

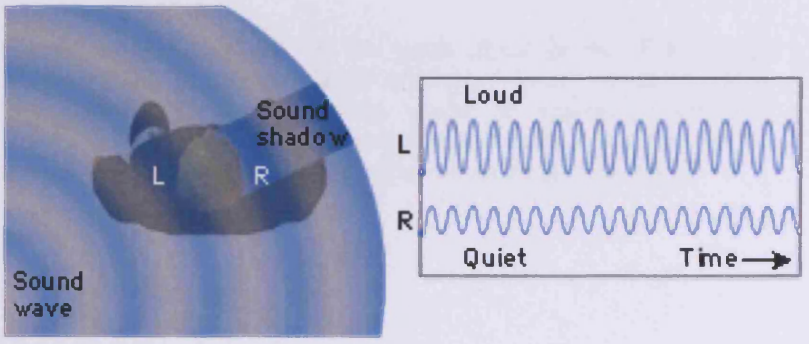


Figure A.2 Differences in the intensity of a wave front at the left and right ear due to the head shadow referred to as the interaural intensity difference (IID) results in a louder sound at the ear nearest the sound source (www.brainconnection.com, Scientific Learning Corporation, 1999).

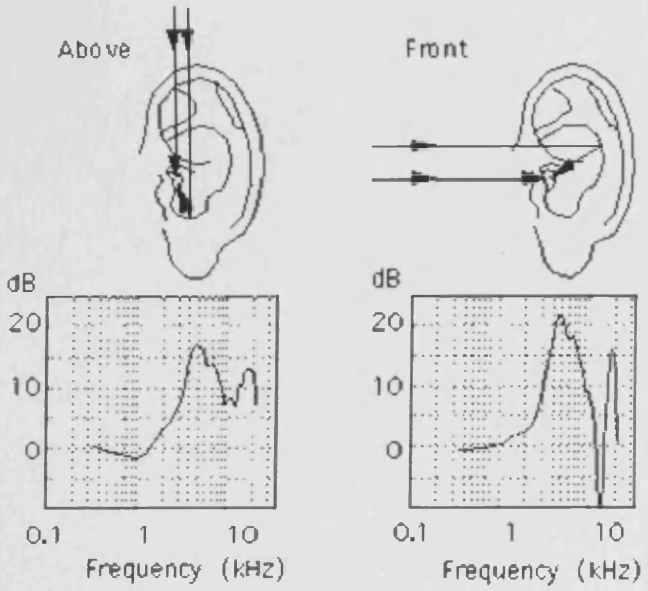


Figure A.3 Convolutions of the pinna result in the directionally-dependent and frequency-specific alterations in the sound spectrum. The resulting spectral cues are useful in resolving directional ambiguities that arise from different spatial locations providing for the same ITDs and/or IIDs (www.emsah.uq.edu.au/linguistics, 2005).

Appendix B

Neural Representation of Sound

The Neural Representation of Sound

The neural basis of sound localization has its foundation in the precise neural coding of the spectral, temporal, and intensity characteristics of a sound arriving at each ear. The subsequent integration of the information from each ear in higher auditory nuclei allows for neural representation of the interaural difference cues essential for sound localization.

The auditory system processes sound in a number of distinct stages. The first stage, neural transduction, involves a degree of pre-processing by the external ear (pinna), and the ear canal, the transformation of the airborne sounds into mechanical vibrations which begins in the middle ear, and the transduction of mechanical events into electrical events in the cochlea of the inner ear. Information from each ear is then processed by a number of auditory nuclei independently, although the signal transformations are thought to be the same for both auditory channels. This stage of processing can be broadly termed monaural processing and incorporates processing by the auditory nerve (AN), cochlear nucleus (CN), and the medial nucleus of the trapezoid body (MNTB). After these stages, information from the left and right ear is integrated in the binaural brainstem nuclei, where a number of signal transformations crucial to neural coding for sound localization cues occur. Binaural signal processing first occurs in nuclei of the superior olivary complex (SOC), followed by nuclei of lateral lemniscus, midbrain, thalamus, and auditory cortex (Figure B.1).

The auditory periphery

When sound interacts with the pinnae, the individual frequency components, particularly high frequencies, are reflected by the convolutions. When the reflected sound enters the ear canal it may do so in any degree of positive or negative phase relationship with other reflections. This will result in either attenuation or amplification of particular frequencies, producing notches and peaks in the sound spectrum reaching the eardrum (Batteau, 1966; Mehrgardt & Mellert, 1977; Shaw, 1966; Shaw & Terenashi, 1968). This 'coloration' of the sound spectrum is unique to a particular location and provides the basis for spectral cues used in determining sound source

elevation and disambiguating sound sources in front from those behind (Kuhn, 1979; Middlebrooks et al. 1989).

The middle ear provides the mechanism for transferring sound waves from air to the fluid-filled cochlea. The middle ear cavity, with its own particular resonant frequency, provides further spectral filtering, amplifying sound around the resonant frequency and effectively damping other frequencies. The eardrum is connected to the cochlea via the middle ear bones (ossicles). The ossicles provide the mechanical advantage necessary to transfer sound waves from an air to a fluid environment, as is the case from the ear drum to the oval window of the cochlea (Figure B.2, A). The movement of the ossicles following the frequency of the stimulating sound wave results in a travelling wave in the fluid of the cochlea.

The cochlea is a coiled, fluid-filled chamber divided into three compartments by Reissner's membrane and the basilar membrane (BM). The three compartments of the cochlear are the scala vestibuli, scala media, and the scala tympani. The BM, which runs from base to apex of the coiled cochlea separating the scala media and scala tympani, is a tapered membrane of varying elasticity, being relatively stiff with less mass at its basal end and relatively less stiff with more mass at its apical end. The variations in elasticity and mass mean the frequency the BM responds to best changes along its length. A travelling wave generated by the ossicles reaches its peak at the location on the BM with the same resonant frequency (Figure B.3). The representation of sound as its component frequencies in a tonotopic gradient established at the level of the BM is a feature of signal processing maintained throughout the auditory system.

The force required to set the basilar membrane in motion combined with the various resonances of the cochlear means there is a frequency- and amplitude-dependent compression of the acoustic waveform (Sumner et al. 2001).

Sitting on top of the basilar membrane is the organ of Corti which contains the sensory hair cells (Figure B.2, B). The inner-hair cells (IHCs) are the site of neural transduction while the outer-hair cells (OHCs) are considered to be involved in active sound amplification (Holley, 1996). Each IHC synapses with a number of afferent fibres of

the auditory nerve while the OHCs also receive efferent input from higher auditory structures via the olivocochlear bundle (Guinan Jr. 1996).

The IHCs are flask-shaped cells with a flat apical surface from which sterocilia protrude. The apical surface of the cell is in contact with the potassium (K^+) rich endolymph of the scala media while the basal part of the hair cell is surrounded by perilymph. In response to vibration of the basilar membrane, the stereocilia of the hair cells deflect from side to side due to the transverse motion of the hair cells toward and away from scala tympani and scala vestibuli (Patuzzi, 1996; Geisler, 1998 pp 80-86; Figure B.2, B).

Deflection of the sterocilia in one direction results in hyperpolarization of the IHC, whilst deflection in the other direction results in depolarization. Depolarization of IHCs occurs due to an influx of K^+ ions via mechanically-gated channels in the sterocilia that raise the resting potential of the cell. This causes calcium-mediated release of excitatory neurotransmitter into synapses located at the basal end of the IHC (Kros, 1996; Giesler, 1998 pp 91-118). Measurement of the IHC receptor potential shows that depolarization of IHCs is synchronised with the peaks in the stimulus waveform. As the frequency of the stimulus increases, the minimum voltage (d.c.) of the receptor potential also increases and the synchronization of the IHC receptor potential to the stimulus waveform decreases. In the guinea pig the ability to follow the stimulus waveform declines from 600 Hz and is absent in response to stimulus frequencies greater than 3.5-kHz (Palmer & Russell, 1986).

Vibration of the basilar membrane also results in OHC depolarization. In response they alter their length and the stereocilia create downward force on the BM. It has been postulated that such changes to the physical properties of the BM amplify sound frequencies that the BM is most responsive to at that location. This active amplification process serves to increase the frequency selectivity of the IHCs established by the tonotopic response characteristics of the BM (Guinan, Jr. 1996).

Neural transduction by the IHC results in substantial changes to the acoustical waveform. The movement of the sterocilia in response to the stimulus waveform results in half-wave rectification because depolarization of the IHCs only occurs during the

positive phase of the acoustic waveform. The frequency selectivity or band-pass filtering characteristics of the BM are sharpened by the mechanical action of the OHCs; the IHCs respond to a narrower range of frequencies than the BM (Patuzzi, 1996). Finally, temporal information conveyed in the fine structure of the acoustic waveform is lost for high frequencies, the upper limit of which, imposed by the mechanical and neural limitations of the cochlea, are species specific.

The auditory nerve (AN) conveys neural information transduced by the hair cells concerning the individual frequencies and their respective amplitudes comprising the acoustic waveform as modified by the cochlear. The AN is comprised of two morphologically and functionally distinct nerve fibres, type I and type II primary auditory neurons. Type I AN fibres receive their main input from the IHCs the dendrites of any one fibre innervating only a single IHC. Type II neurons synapse with the OHCs and are thought to have a role in determining the response properties of type I fibres. Both types of fibres also receive input from the SOC (Rugerro, 1992; Rouiller, 1997).

The responses of Type I AN fibres are largely determined by response properties of the IHC with which they synapse. The frequency filtering properties of Type I AN fibres are described by their tuning curves, the threshold of their response to tones of varying frequency. Such tuning curves are 'V' shaped when responses are plotted as a function of frequency (abscissa) and level (ordinate). The frequency-filtering characteristics of the fibre are determined by the location of the IHC it innervates on the basilar membrane (Rouiller, 1997). In response to tonal stimuli the discharges of some Type I AN fibres are synchronised with a particular phase of the acoustic wave form (phase locking). In the guinea pig the upper limits of AN nerve fibre phase locking are determined by capacity of the IHCs to follow the fine structure of the acoustic wave form (Palmer & Russell, 1986). The accurate representation of the temporal information in the acoustic waveform for these frequencies is essential to the neural representation of ITDs. For continuous stimuli the responses of AN fibres decrease to a steady state. This adaptation of AN responses is thought to be largely due to depletion of excitatory neurotransmitter at the IHC synapse (Meddis, 1986; Geisler, 1998 pp 169-197; see Figure B.4 for an example of AN output).

Monaural Auditory Nuclei

The cochlear nucleus (CN) located in the mammalian brainstem is the first nucleus in the monaural auditory pathway, and is the first stage of auditory processing where the responses of neurons show a substantial departure from simple representation of the peripherally-filtered acoustic waveform. The CN is comprised of three distinct sub nuclei; the anteroventral and posteroventral divisions (AVCN & PVCN), and the dorsal cochlear nucleus (DCN). Fibres of the AN bifurcate at the CN into an anterior and posterior branch projecting to all three areas (Cant, 1992; Romand & Avan, 1997). AN fibres receiving inputs from IHCs located at the base of the cochlea (high frequency) project to dorsal CN while those with inputs originating near the apex of the cochlea (low frequency) project to ventral CN establishing a tonotopic gradient of layers of neurons most responsive to particular frequencies (Cant, 1992).

The division of the CN into sub-nuclei can be justified based on cell morphology as well as functional specialisation of the neurons in those areas for particular acoustic tasks. Each sub-division is comprised of morphologically-distinct cell types that respond with distinct temporal patterns to the same stimuli not necessarily maintaining the response characteristics of AN fibres (Rhode & Greenberg, 1992). The majority of neurons located in the AVCN, and to a lesser extent, the PVCN have responses most similar to their AN counterparts (Rhode & Greenberg, 1992). It is neurons located in these divisions of the CN that provide the precisely-timed (phase-locked) input to binaural auditory nuclei required for auditory processing of ITD cues. These neurons also project to binaural nuclei that process IID cues. The neurons of the DCN exhibit responses most divergent from those of the AN although such responses are also seen in the PVCN and AVCN to a lesser extent (Rhode & Greenberg, 1992; Romand & Avan, 1997). The combination of intrinsic and extrinsic inhibitory circuitry and intrinsic excitatory circuitry, in addition to the variety of cell types found in the CN are thought to contribute to the modification of the response characteristics of the neurons from those observed in AN fibres.

The subdivisions of the CN project to distinct groups of auditory nuclei via the ventral acoustic stria (from AVCN), the intermediate acoustic stria (from PVCN) and the dorsal acoustic stria (from DVCN) resulting in parallel pathways of auditory processing (Cant, 1992). The AVCN has solely excitatory projections terminating in the ipsilateral

lateral superior olive (LSO), medial superior olive (MSO), lateral nucleus of the trapezoid body (LNTB), periolivary nuclei of the SOC and the DCN (Romand & Avan, 1997). The contralateral excitatory projections terminate in the MSO, the ventral and medial nuclei of the trapezoid body (VNTB, MNTB), ventral nucleus of lateral lemniscus (VNLL) and the inferior colliculus (IC; Romand & Avan, 1997). The AVCN also has excitatory local circuitry. The majority of neurons that project to the contralateral MSO from AVCN have the temporal response characteristics of the AN fibres that innervate them, showing similar frequency selectivity and exhibiting phase-locked responses to tonal stimuli essential in binaural processing of ITD cues (Rhode & Greenberg, 1992; Romand & Avan, 1997).

Neurons of the PVCN utilise both excitatory and inhibitory neurotransmitters. Ipsilateral excitatory projections from the PVCN terminate in the periolivary nuclei. Contralateral excitatory projections terminate in the periolivary nuclei, the VNLL and the IC. The PVCN has a glycinergic inhibitory projection to the ipsilateral and contralateral periolivary nuclei. There is also excitatory local circuitry within the PVCN (Cant, 1992).

Neurons of the DCN project mainly within the CN providing much of the inhibitory input found in the CN utilizing γ-aminobutyric acid (GABA) and glycine as inhibitory neurotransmitters. GABA-ergic and glycinergic terminations originating in the DCN are found in the AVCN, PVCN as well as locally. The DCN also provides excitatory input to neurons within the DCN and to the contralateral IC and VNLL (Cant, 1992).

In addition to the intrinsic inhibitory inputs originating in the DCN and PVCN neurons of the CN, also receives substantial inhibitory inputs from other auditory nuclei. The VNTB, LNTB and periolivary nuclei of the SOC are thought to be the external source of both GABA-ergic and glycinergic inhibition to the CN (Cant, 1992; Romand & Avan, 1997).

Binaural Auditory Nuclei

The superior olivary complex (SOC), located in the mammalian brainstem, includes the medial nucleus of the trapezoid body (MNTB) the lateral superior olive (LSO) the

medial superior olive (MSO) and associated peripheral nuclei. The SOC is the site of primary binaural integration in the auditory system. The MNTB is a monaural nucleus, the neurons of which serve largely as interneurons for ascending input from the contralateral CN (Helfert & Aschoff, 1997). Neurons in the MSO and LSO are the first to exhibit interaural difference processing.

Neurons of the MNTB receive the majority of their input from the contralateral CN with the primary function of the MNTB to provide precisely-timed inhibitory input to the ipsilateral LSO and MSO (Schwartz, 1992; Helfert & Aschoff, 1997). Most neurons in the MNTB have response properties that are described by the responses of AN fibres reflecting the nature of the major excitatory input to the nucleus, the VCN (Helfert & Aschoff, 1997). Projections from the MNTB are glycinergic and tonotopically organised terminating in the ipsilateral MSO, LSO, periolivary nuclei, intermediate nucleus of lateral lemniscus (INLL), and to a lesser extent the ventral nucleus of lateral lemniscus (VNLL). The MNTB also has bilateral projections terminating in the VCN (Helfert & Aschoff, 1997).

Neurons from the LSO integrate neural output from the left and right monaural auditory pathways resulting in responses characteristics that are primarily determined by the IID of the stimulus. The LSO is comprised mainly of neurons with homogenous morphology and response characteristics (Swartz, 1992; Ortel & Wickesberg, 1996; Helfert & Aschoff, 1997). The major input to the LSO originates in the ipsilateral MNTB and VCN with additional input coming from the ipsilateral and contralateral periolivary nuclei (Helfert & Aschoff, 1997). The input from the VCN is excitatory and stimulation of the majority of LSO neurons by presenting sounds to the ipsilateral ear results in an intensity-dependent increase in response. The input from the MNTB is inhibitory (glycinergic), and because neurons located in the MNTB serve largely as interneurons for the contralateral CN stimulation of LSO neurons by presenting sound to the contralateral ear results in a decrease in their response. Therefore the output of LSO neurons is determined by the relative levels of the sound at each ear with changes in the response representing changes in the IID of the stimulus. Neurons from the LSO provide tonotopically organised excitatory input to the contralateral IC, DNLL and VNLL and glycinergic inhibitory input to the ipsilateral IC (Helfert & Aschoff, 1997).

The MSO is the brainstem nucleus responsible for processing ITDs. Neurons of the MSO receive excitatory input from the ipsilateral and contralateral AVCN and inhibitory input from neurons located in the ipsilateral MNTB and LNTB (Swartz, 1996). The major outputs of the MSO are excitatory and terminate bilaterally in the IC and ipsilaterally in the DNLL (Swartz, 1996; Helfert & Aschoff, 1997).

The output of low-BF MSO neurons depends on the ITD of the stimulus. Such neurons receive precisely-timed, phase locked inputs from the ipsilateral and contralateral AVCN. When presented with tones to either the ipsilateral or contralteral ear alone, the output of MSO neurons is locked to a particular phase angle of the sine wave (Helfert & Aschoff, 1997). The difference in preferred phase angle between the two ears can be described as a difference in the phase angle to which the response of the neuron is locked when sound is delivered binaurally (Goldberg & Brown, 1969). The neuron responds best to binaural tones when this phase difference is compensated for by an ITD imposed on binaurally presented sound, known as the neurons best ITD.

Jeffress (1948) first proposed a model describing the auditory system based on a mechanism of binaural coincidence detection that is analogous to the response characteristics of MSO neurons. Jeffress proposed an array of coincidence detectors, each tuned to a preferred ITD, to encode for all spatial locations by means of an explicit local code. Each coincidence detector was rendered sensitive to a preferred ITD by means of a compensating difference in neuronal path length from each ear. When this difference was exactly compensated by the ITD arising from a sound source having a particular position in space, neural signals arriving from each ear would coincide in a binaural coincidence detector, causing it to fire maximally (Figure B.5). The pattern of activity across the array of coincidence detectors represented the spatial location of the sound. While this hypothesis has been anatomically validated in the barn owl, evidence from mammals is more equivocal. Brand et al. (2002) have shown ITD tuning of individual MSO neurons of the gerbil is created through inhibitory input (glycinergic) from the ipsilateral MNTB. When glycinergic input is blocked locally in the MSO, neural tuning for ITD is shifted from the preferred ITD to zero. This suggests, that in mammals, the delay lines proposed by Jeffress may be implemented through inhibitory mechanisms rather than differences in axonal conduction time. While the mechanism behind the ITD selectivity of neurons may vary across species, computational modeling

of the auditory system behaving as a mechanism that cross correlates the neural activity from the monaural auditory pathways in a manner analogous to coincidence detection has yielded accurate predications of behaviour (Stern, 1988; Trahiotis et al. 2001).

The ascending afferent fibres from the lower auditory system to the midbrain form part of the tract of nerve fibres the lateral lemniscus (LL). Amongst the LL are a number of morphologically distinct nuclei two of which are the ventral nucleus of lateral lemniscus (VNLL) and the dorsal nucleus of lateral lemniscus (DNLL).

Neurons of the VNLL receive their major excitatory input from the contralateral VCN and as such respond mainly to stimulation of the contralateral ear (Swartz, 1992). There are also minor inhibitory inputs originating in the ipsilateral MNTB and periolivary nuclei (Swartz, 1992). Neurons of the VCN vary in their response characteristics some showing sharp frequency tuning others very broad frequency tuning. The timing of responses with the waveform of stimuli is very precise to both the onset of the stimulus and the ongoing temporal pattern of the waveform (Romand & Avan, 1997). Due to this feature it has been speculated that the function of the VNLL is to analyse amplitude modulations present in such sounds as animal vocalisations (Ortel & Wickesberg, 2002). Neurons of the VNLL project predominately to the ipsilateral IC the nature of the projection being inhibitory (glycinergic; Oliver & Huerta, 1992).

The DNLL is a binaural nucleus located at the level of the brainstem that provides the major inhibitory input to the IC. Due to the binaural response characteristics of neurons located in the DNLL it has the capacity to provide directionally dependent inhibition that may be useful for binaural auditory tasks such as suppression of interaural cues present in reverberated sound (Pollack et al. 2002).

The DNLL receives bilateral excitatory projections from the AVCN, a contralateral excitatory projection from the LSO and an ipsilateral excitatory input from the MSO (Swartz, 1992; Helfert & Aschoff, 1997). Inhibitory input to the DNLL originates in the ipsilateral LSO (glycinergic), and the contralateral DNLL. There are also lesser inputs originating from the ipsilateral IC and VNLL (Swartz, 1992; Helfert & Aschoff, 1997). Neurons of the DNLL are tonotopically organised although in the guinea pig such organisation is concentric rather than in a dorsoventral gradient (low to high) as in

other auditory nuclei. The majority of DNLL neurons are binaural and show response characteristics that are modulated by the ITD and ILD of a stimulus. The major outputs of the DNLL terminates in the contralateral DNLL and bilaterally in the IC and are exclusively inhibitory (GABA-ergic; Oliver, & Huerta, 1992).

The inferior colliculus (IC) is the major auditory nucleus in the midbrain. It receives direct ascending input from monaural and binaural auditory nuclei, as well as descending input from the thalamus and cortex (Oliver & Huerta, 1992; Ehert, 1997). The inputs to the IC allow the possibility of transfer of information from lower auditory nuclei to higher auditory centres and vice versa as well as novel auditory processing due to the integration of inputs from multiple auditory nuclei both higher and lower in the system in a single neuron. The IC also provides input to brain nuclei that integrate information from a number of sensory modalities that contribute to motor control. In addition the IC is considered to be integral to the processing of sound localization cues in reverberant environments (Yin, 1994; Fitzpatrick et al. 1995; Pollack et al. 2002).

The IC is comprised of several morphologically and functionally unique regions whose predominant inputs originate from distinct auditory nuclei. The IC has been divided into the dorsal cortex of the IC (ICdc), the external nucleus of the IC (ICx), the central nucleus of the IC (ICc) and the peri central nucleus (ICp) of the IC on the basis of cell type, laminar structure of dendritic fields and afferent inputs (Aitkin, et al. 1974; Syka et al. 2000). The ICdc is located on the dorsal surface of the IC, the ICx is located on the lateral edge of the IC extending in a dorsal to ventral direction, the ICc is located medially and extends laterally to the ICx and ventrally while the ICp is located ventrally and on the anterior surface of the ICc. There is generally a tonotopic gradient in a dorsal to ventral direction of low-BF to high-BF neurons within all regions, although the degree of tonotopy of neurons located within a layer varies between the areas. The tonotopic gradient is most defined in the ICc (Semple & Aitkin, 1979; Aitkin & Moore, 1974).

Neurons of the ICc receive monaural inputs from all regions of the contralateral CN and from the ipsilateral VNLL and INLL. Inputs from the AVCN and PVCN are most likely excitatory while inputs from the DCN (GABA-ergic) are thought to be inhibitory, as are those from the INLL and VNLL (GABA-ergic & glycinergic; Oliver

& Huerta, 1992). Inputs from the VCN terminate more ventrally in the ICc while those from DCN terminate more dorsally predominately in the ICdc. Inputs from the INLL and VNLL terminate in the ventral areas of ICc. Binaural inputs originating in the ipsilateral and contralateral MSO terminate predominantly in the dorsolateral ICc (Oliver & Huerta, 1992). Excitatory input from the contralateral LSO terminates in more ventral areas of ICc, while inhibitory (glycinergic) input from the ipsilateral LSO terminates more ventrally and laterally (Oliver & Huerta, 1992). Bilateral GABA-ergic inhibitory input originating from the DNLL terminates throughout the IC. Descending input from the contralateral and ipsilateral Aud. C terminates primarily in the ICdc and ICx as does the input from the thalamus and SC. There is also excitatory input originating from the contralateral IC and extensive intrinsic circuitry (Oliver & Huerta, 1992).

The responses of IC neurons vary depending on their location within the IC reflective of the various inputs that predominate in these regions. As such neurons of the IC exhibit response characteristics seen in neurons located in lower binaural and monaural auditory nuclei, as well as novel response characteristics resulting from the integration of inputs. Neurons outside of the ICc have broader tuning curves and higher sound level thresholds at BF than those in the ICc and are not as sensitive to interaural difference cues as the neurons of the ICc (Irvine, 1992; Ehert, 1997). Therefore the ICc is the region best equipped to process sound localization cues. The majority of neurons within the ICc respond to a tone burst with a either, a higher probability of spikes to the onset of the tone, subsequently decaying over the duration of the tone (phasic response), or with a relatively constant number of spikes throughout the duration of the tone (tonic response; Irvine, 1992; Casseday et al. 2002). However, a great variety of other response types are seen in ICc neurons to a lesser extent such as neurons that respond only to the onset or offset of a tone (Irvine, 1992). Low-BF IC neurons often exhibit phase locking and are sensitive to the ITD of a tone In contrast high-BF neurons located ventrally show sensitivity to the IID of a tone and in some cases to the onset ITD (Semple & Aitkin, 1979). Such binaural sensitivity is thought be the result of processing in the MSO and LSO and the resulting excitatory projections as well as being generated anew in the IC (Irvine, 1992; Ehert, 1997). The inhibitory input arising from the DNLL and ipsilateral LSO and VNLL is thought to provide inhibitory input to

such neurons that in bats has proved to be involved in the processing of sound under acoustically reverberant listening conditions (Zhou & Jen, 2004).

The main output of the IC is to the Aud. C via the Thalamus and the cortico-collicular pathway and to lower auditory centres via the cortico-fugal pathway providing feedback from the cortex as early in auditory processing as at the level of the OHCs of the cochlear. Output to the SC and cerebellum via the fugo-tectal pathway provides direct control of motor coordination in response to auditory stimulation. Thus, the IC is the auditory centre through which all aspects of auditory processing are represented and mediated.

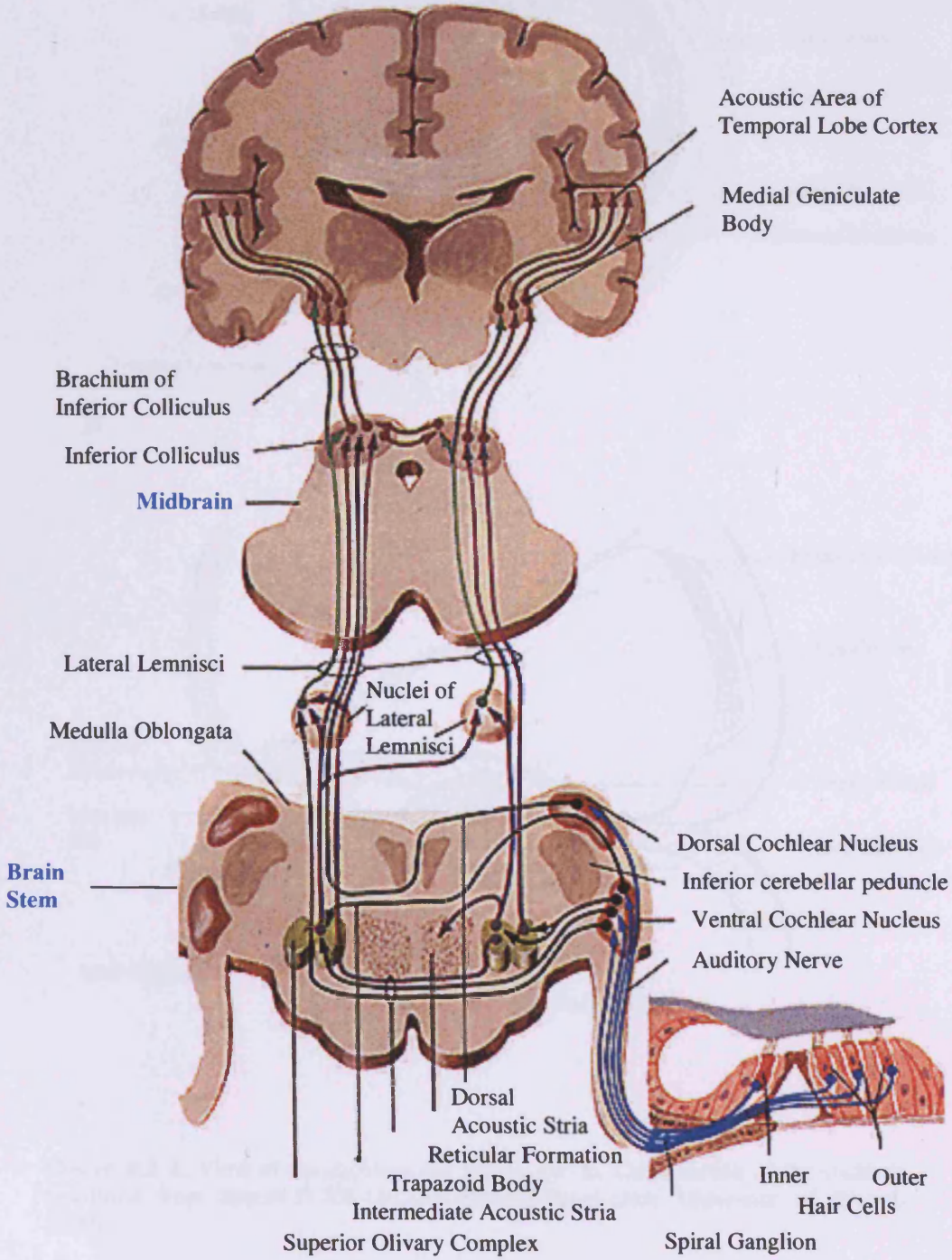


Figure B.1 The structure of the mammalian auditory system indicating major auditory nuclei (adapted from the CIBA collection of medical illustrations volume 1).

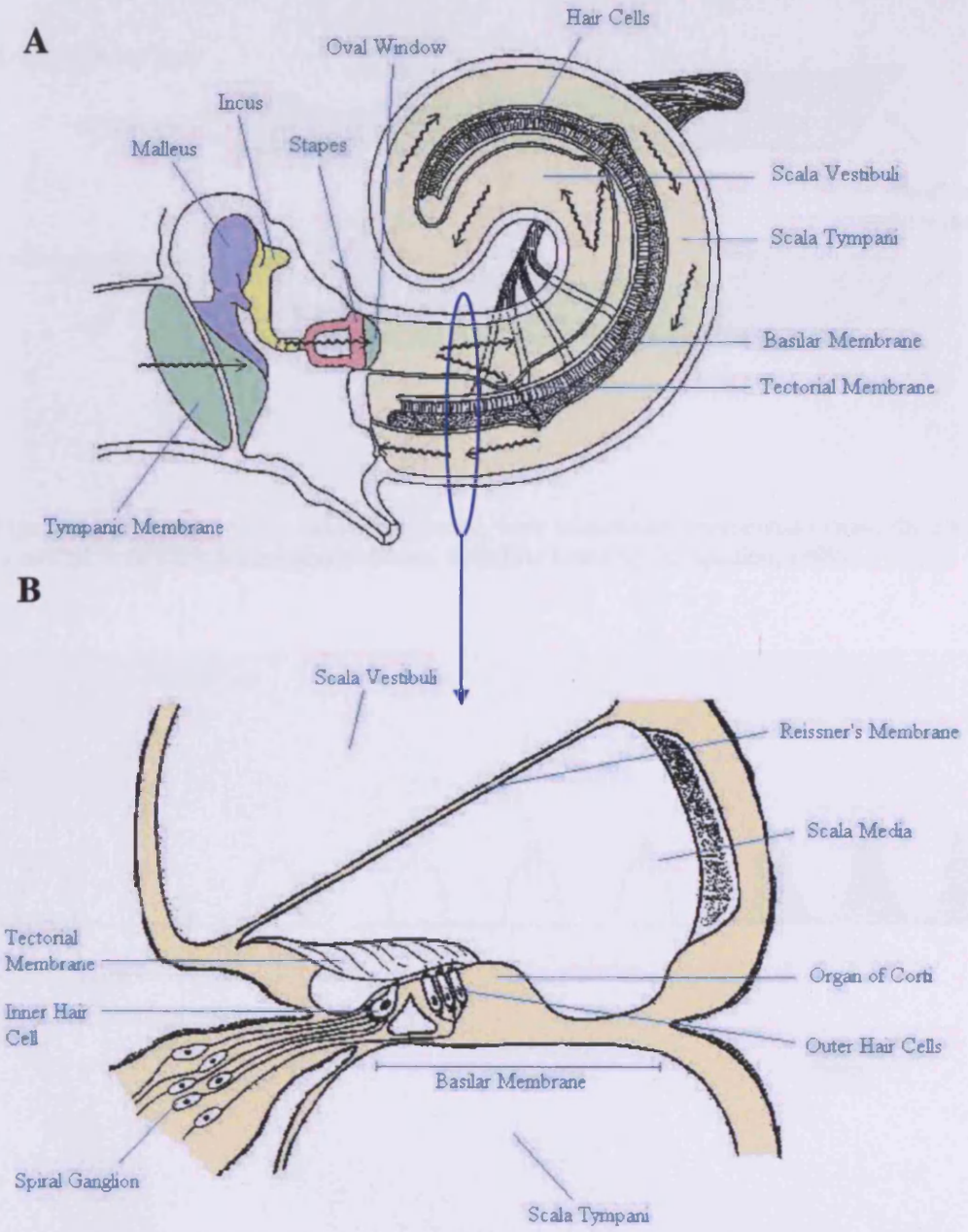


Figure B.2 A. View of the cochlear and middle ear. B. Cross section of the cochlear (modified from http.//137.222.110.150/calnet/Aud/page2.htm University of Bristol, 2000).

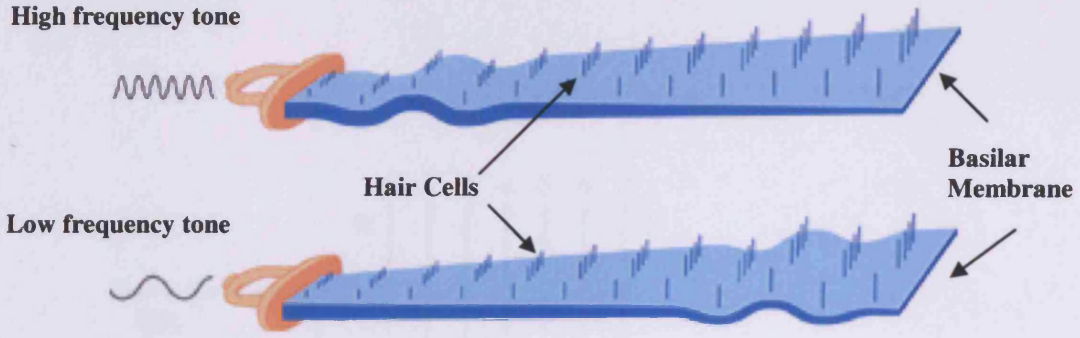


Figure B.3 Tonotopy of the basilar membrane. Low frequencies represented toward the apex (modified from www.brainconnection.com, Scientific Learning Corporation, 1999).

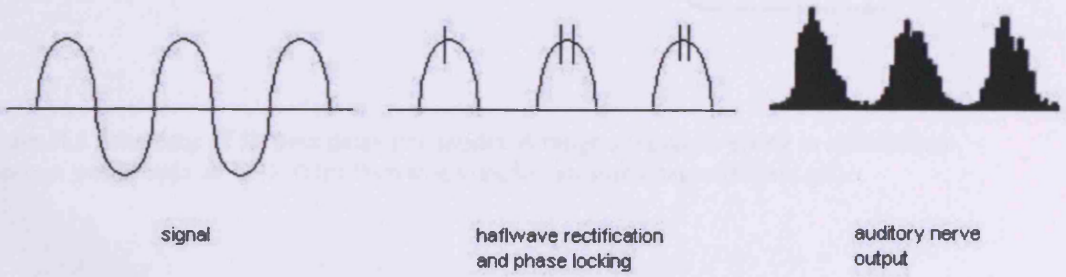


Figure B.4 Transformation in signal by auditory periphery. Half wave rectification and phase locking occurring in the cochlear and resulting compressed output of auditory nerve

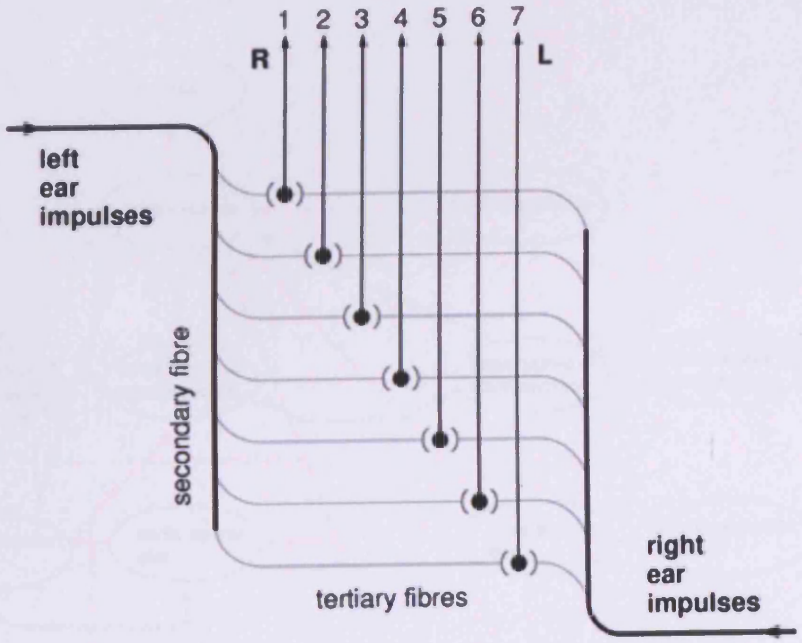


Figure B.5 Schematic of Jeffress delay line model. A range of neurons acting as coincidence detectors would code all ITDs (http://www.aes.org/sections/uk/images/jeffress.gif).

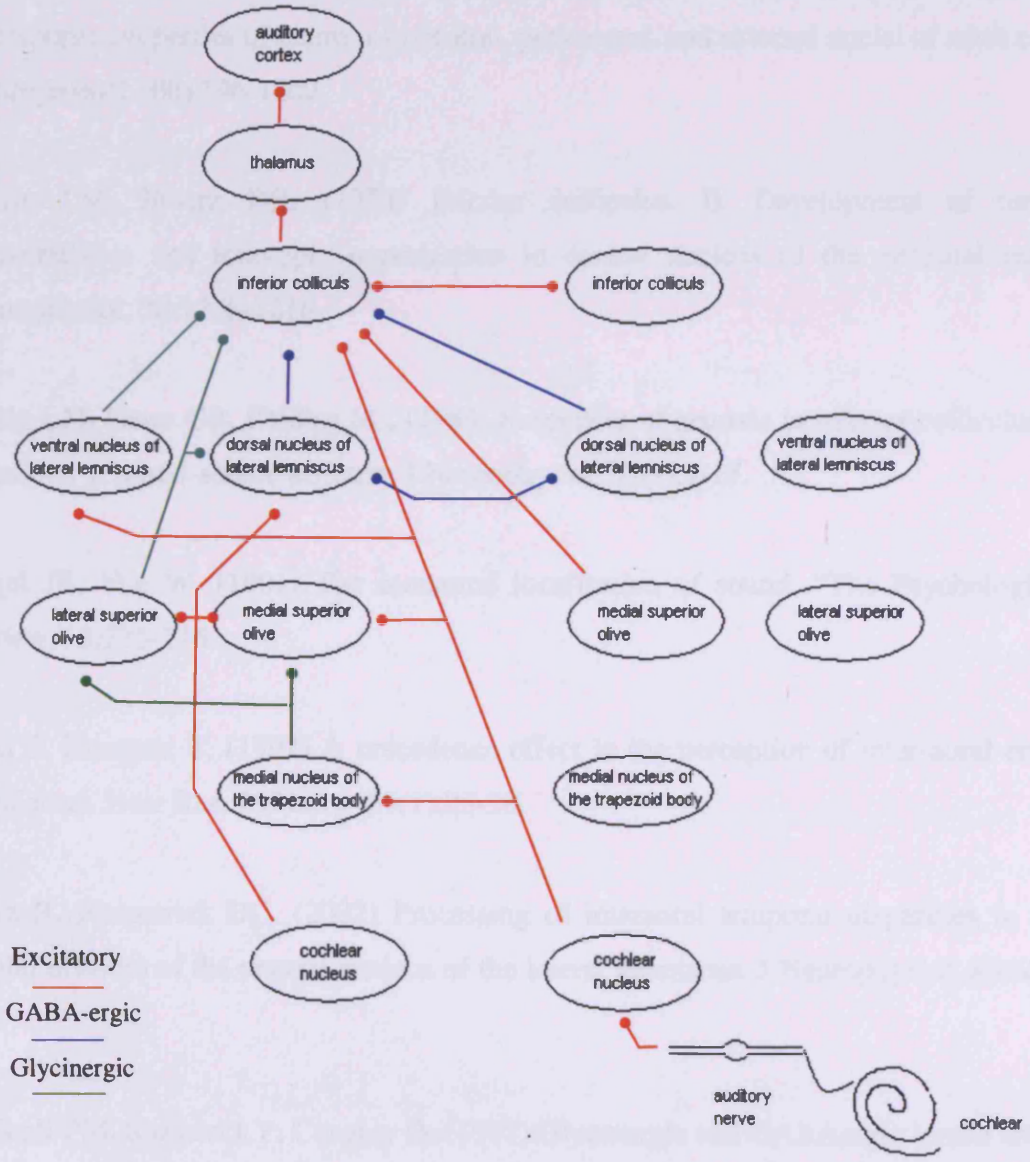


Figure B.6 Schematic representation major of ascending afferent and efferent connections of the mammalian auditory pathway.

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